

PCTWORLD INTELLECTUAL PROPERTY ORGANIZATION
International Bureau

INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification ⁷ : C12N 15/10, C07K 14/47, C12N 9/00, C07K 14/705		A2	(11) International Publication Number: WO 00/53742 (43) International Publication Date: 14 September 2000 (14.09.00)
(21) International Application Number: PCT/US00/06280		(81) Designated States: AE, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CR, CU, CZ, DE, DK, DM, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW, ARIPO patent (GH, GM, KE, LS, MW, SD, SL, SZ, TZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG).	
(22) International Filing Date: 9 March 2000 (09.03.00)			
(30) Priority Data: 60/123,667 9 March 1999 (09.03.99) US Not furnished 8 March 2000 (08.03.00) US			
(63) Related by Continuation (CON) or Continuation-in-Part (CIP) to Earlier Application US 60/123,667 (CIP) Filed on 9 March 1999 (09.03.99)			
(71) Applicant (for all designated States except US): CURAGEN CORPORATION [US/US]; 555 Long Wharf Drive, 11th floor, New Haven, CT 06511 (US).			
(72) Inventor; and (75) Inventor/Applicant (for US only): SHIMKETS, Richard, A. [US/US]; 191 Leete Street, West Haven, CT 06516 (US).			
(74) Agent: ELRIFI, Ivor, R.; Mintz, Levin, Cohn, Ferris, Glovksy and Popeo, P., C., One Financial Center, Boston, MA 02111 (US).			
(54) Title: POLYNUCLEOTIDES AND PROTEINS ENCODED THEREBY			
(57) Abstract			
<p>The present invention provides novel isolated SECX polynucleotides and the membrane-associated or secreted polypeptides encoded by the SECX polynucleotides. Also provided are the antibodies that immunospecifically bind to a SECX polypeptide or any derivative, variant, mutant or fragment of the SECX polypeptide, polynucleotide or antibody. The invention additionally provides methods in which the SECX polypeptide, polynucleotide and antibody are utilized in the detection and treatment of a broad range of pathological states, as well as to other uses.</p>			

FOR THE PURPOSES OF INFORMATION ONLY

Codes used to identify States party to the PCT on the front pages of pamphlets publishing international applications under the PCT.

AL	Albania	ES	Spain	LS	Lesotho	SI	Slovenia
AM	Armenia	FI	Finland	LT	Lithuania	SK	Slovakia
AT	Austria	FR	France	LU	Luxembourg	SN	Senegal
AU	Australia	GA	Gabon	LV	Latvia	SZ	Swaziland
AZ	Azerbaijan	GB	United Kingdom	MC	Monaco	TD	Chad
BA	Bosnia and Herzegovina	GE	Georgia	MD	Republic of Moldova	TG	Togo
BB	Barbados	GH	Ghana	MG	Madagascar	TJ	Tajikistan
BE	Belgium	GN	Guinea	MK	The former Yugoslav Republic of Macedonia	TM	Turkmenistan
BF	Burkina Faso	GR	Greece			TR	Turkey
BG	Bulgaria	HU	Hungary	ML	Mali	TT	Trinidad and Tobago
BJ	Benin	IE	Ireland	MN	Mongolia	UA	Ukraine
BR	Brazil	IL	Israel	MR	Mauritania	UG	Uganda
BY	Belarus	IS	Iceland	MW	Malawi	US	United States of America
CA	Canada	IT	Italy	MX	Mexico	UZ	Uzbekistan
CF	Central African Republic	JP	Japan	NE	Niger	VN	Viet Nam
CG	Congo	KE	Kenya	NL	Netherlands	YU	Yugoslavia
CII	Switzerland	KG	Kyrgyzstan	NO	Norway	ZW	Zimbabwe
CI	Côte d'Ivoire	KP	Democratic People's Republic of Korea	NZ	New Zealand		
CM	Cameroun	KR	Republic of Korea	PL	Poland		
CN	China	KZ	Kazakhstan	PT	Portugal		
CU	Cuba	LC	Saint Lucia	RO	Romania		
CZ	Czech Republic	LI	Liechtenstein	RU	Russian Federation		
DE	Germany	LK	Sri Lanka	SD	Sudan		
DK	Denmark	LR	Liberia	SE	Sweden		
EE	Estonia			SG	Singapore		

POLYNUCLEOTIDES AND PROTEINS ENCODED THEREBY

FIELD OF THE INVENTION

The invention relates to polynucleotides and secreted or membrane-associated polypeptides
5 encoded by such polynucleotides, as well as vectors, host cells, antibodies and recombinant methods
for producing the polypeptides and polynucleotides.

BACKGROUND OF THE INVENTION

Eukaryotic cells are subdivided by membranes into multiple functionally distinct
compartments that are referred to as organelles. Each organelle includes proteins essential for its
10 proper function. These proteins can include sequence motifs often referred to as sorting signals. The
sorting signals can aid in targeting the proteins to their appropriate cellular organelle(s). In addition,
sorting signals can direct some proteins to be exported, or secreted, from the cell.

One type of sorting sequence is a signal sequence (also referred to as a signal peptide or leader
sequence). The signal sequence is present as an amino-terminal extension on a newly synthesized
15 polypeptide chain. A signal sequence targets proteins to an intracellular organelle called the
endoplasmic reticulum (ER).

The signal peptide takes part in an array of protein-protein and protein-lipid interactions that
result in translocation of a polypeptide containing the signal sequence through a channel in the ER.
After translocation, a membrane-bound enzyme (signal peptidase) liberates the mature protein from
20 the signal sequence.

The ER functions to separate membrane-bound proteins and secreted proteins from proteins
that remain in the cytoplasm. Once targeted to the ER, both secreted and membrane-bound proteins
can be further distributed to another cellular organelle called the Golgi apparatus. The Golgi directs
the proteins to vesicles, lysosomes, the plasma membrane, mitochondria and other cellular organelles.

25 Only a limited number of genes encoding human membrane-bound and secreted proteins have
been identified. Examples of known secreted proteins include human insulin, interferon, interleukins,
transforming growth factor-beta, human growth hormone, erythropoietin, lymphokines. A need exists
for identifying and characterizing additional novel human secreted proteins and the genes that encode
them.

30

SUMMARY OF THE INVENTION

The present invention is based, in part, upon the discovery of novel human polynucleotide
sequences and the membrane-bound or secreted polypeptides encoded by these sequences.

Polypeptides of the invention include a chemokine receptor-like protein (clone 2777610), semaphorin protein-like splice variants (assembled clones 2864933-1 and 2864933-2, and the pCEP4/Sec-2864933 vector and cDNA clone pCR2.1-2864933), a putative mitochondrial protein (clone 2982339), SLIT protein-like splice variants (assembled clones 3352358-1 and 3352358-2 and the cDNA clone 5 3352358-S153A), a putative microbody (peroxisome) associated protein (clones 3884846, 3884846-1 and 3884846-2), a tetraspanin-like protein (clones 3911675 and 3911675-2), a putative proline-rich membrane protein (clones 4004056 and 4004056.0.143u), a laminin β-chain precursor-like protein (clone 4004731-1), AVENA protein-like splice variants (clones 4009334-1 and 4009334-2), a fetal lung-associated protein (clone 4035508) and a myeloid upregulated protein (clone 4339264). These 10 polynucleotides and the polypeptides encoded thereby are collectively referred to as the SECX gene set, the sequences of which are disclosed in SEQ ID NOS:1-32.

In one aspect, the invention includes an isolated SECX nucleic acid molecule which includes a nucleotide sequence encoding a polypeptide that includes the amino acid sequence of one or more of SEQ ID NOS:2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22, 24, 26, 28, 30, 32, 75, 77, 79 and 81. For example, 15 in various embodiments, the nucleic acid can include a nucleotide sequence that includes SEQ ID NOS:1, 3, 5, 7, 9, 11, 13, 15, 17, 19, 21, 23, 25, 27, 29, 31, 74, 76, 78 and 80. Alternatively, the encoded SECX polypeptide may have a variant amino acid sequence, e.g., have an identity or similarity less than 100% to the disclosed amino acid sequences, as described herein.

The invention also includes an isolated polypeptide that includes the amino acid sequence of 20 one or more of SEQ ID NOS 2, 4, 6, 8, 10, 12, 14, 16, 20, 22, 24, 26, 28, 30, 32, 34, 36, 38, 40, 42, 44, 46, or 48; or a fragment having at least 15 amino acids of these amino acid sequences. Also included is a naturally occurring polypeptide variant of a SECX polypeptide, wherein the polypeptide is encoded by a nucleic acid molecule which hybridizes under stringent conditions to a nucleic acid molecule consisting of a SECX nucleic acid molecule.

25 Also included in the invention is an antibody which selectively binds to a SECX polypeptide.

The invention further includes a method for producing a SECX polypeptide by culturing a host cell expressing one of the herein described SECX nucleic acids under conditions in which the nucleic acid molecule is expressed.

The invention also includes methods for detecting the presence of a SECX polypeptide or 30 nucleic acid in a sample from a mammal, e.g., a human, by contacting a sample from the mammal with an antibody which selectively binds to one of the herein described polypeptides, and detecting the formation of reaction complexes including the antibody and the polypeptide in the sample. Detecting the formation of complexes in the sample indicates the presence of the polypeptide in the sample.

The invention further includes a method for detecting or diagnosing the presence of a disease, e.g., a pathological condition, associated with altered levels of a polypeptide having an amino acid sequence at least 80% identical to a SECX polypeptide in a sample. The method includes measuring the level of the polypeptide in a biological sample from the mammalian subject, e.g., a human, and 5 comparing the level detected to a level of the polypeptide present in normal subjects, or in the same subject at a different time, e.g., prior to onset of a condition. An increase or decrease in the level of the polypeptide as compared to normal levels indicates a disease condition.

Also included in the invention is a method of detecting the presence of a SECX nucleic acid molecule in a sample from a mammal, e.g., a human. The method includes contacting the sample with 10 a nucleic acid probe or primer which selectively hybridizes to the nucleic acid molecule and determining whether the nucleic acid probe or primer binds to a nucleic acid molecule in the sample. Binding of the nucleic acid probe or primer indicates the nucleic acid molecule is present in the sample.

The invention further includes a method for detecting or diagnosing the presence of a disease 15 associated with altered levels of a SECX nucleic acid in a sample from a mammal, e.g., a human. The method includes measuring the level of the nucleic acid in a biological sample from the mammalian subject and comparing the level detected to a level of the nucleic acid present in normal subjects, or in the same subject at a different time. An increase or decrease in the level of the nucleic acid as compared to normal levels indicates a disease condition.

20 The invention also includes a method of treating a pathological state in a mammal, e.g., a human, by administering to the subject a SECX polypeptide to the subject in an amount sufficient to alleviate the pathological condition. The polypeptide has an amino acid sequence at least 80% identical to a SECX polypeptide.

25 Alternatively, the mammal may be treated by administering an antibody as herein described in an amount sufficient to alleviate the pathological condition.

Pathological states for which the methods of treatment of the invention are envisioned include 30 a cancer, e.g., colorectal carcinoma, a prostate cancer a benign tumor, an immune disorder, an immune deficiency, an autoimmune disease, acquired immune deficiency syndrome, transplant rejection, allergy, an infection by a pathological organism or agent, an inflammatory disorder, arthritis, a hematopoietic disorder, a skin disorder, atherosclerosis, restenosis, a neurological disease, Alzheimer's disease, trauma, a surgical or traumatic wound, a spinal cord injury, and a skeletal disorder.

Unless otherwise defined, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this invention belongs. Although methods and materials similar or equivalent to those described herein can be used in the practice or testing of the present invention, suitable methods and materials are described below. All publications, patent applications, patents, and other references mentioned herein are incorporated by reference in their entirety. In the case of conflict, the present specification, including definitions, will control. In addition, the materials, methods, and examples are illustrative only and not intended to be limiting.

Other features and advantages of the invention will be apparent from the following detailed description and claims.

BRIEF DESCRIPTION OF THE DRAWINGS

FIG. 1 is a representation of the nucleotide and encoded polypeptide sequences of clone 2777610.

FIG. 2 is a representation of the nucleotide and encoded polypeptide sequences of clone 15 2864933-1.

FIG. 3 is a representation of the nucleotide and encoded polypeptide sequences of clone 2864933-2.

FIG. 4 is a representation of the nucleotide and encoded polypeptide sequences of clone 20 2982339.

FIG. 5 is a representation of the nucleotide and encoded polypeptide sequences of clone 3352358-1.

FIG. 6 is a representation of the nucleotide and encoded polypeptide sequences of clone 3352358-2.

FIG. 7 is a representation of the nucleotide and encoded polypeptide sequences of clone 25 3884846 (FIG. 7A), clone 3884846-1 (FIG. 7B), and clone 3884846-2 (FIG. 7C).

FIG. 8 is a representation of the nucleotide and encoded polypeptide sequences of clone 3911675 (FIG. 8A) and clone 3911675-2 (FIG. 8B).

FIG. 9 is a representation of the nucleotide and encoded polypeptide sequences of clone 4004056 (FIG. 9A) and clone 4004056.0.143u (FIG. 9B).

FIG. 10 is a representation of the nucleotide and encoded polypeptide sequences of clone 30 4004731-1.

FIG. 11 is a representation of the nucleotide and encoded polypeptide sequences of clone 4009334-1.

FIG. 12 is a representation of the nucleotide and encoded polypeptide sequences of clone 4009334-2.

FIG. 13 is a representation of the nucleotide and encoded polypeptide sequences of clone 4035508.

5 FIG. 14 is a representation of the nucleotide and encoded polypeptide sequences of clone 4339264.

FIG. 15 is a representation of the nucleotide and encoded polypeptide sequences of the cDNA clone pCR2.1-2864933.

10 FIG. 16 depicts the Western blot after reducing SDS-PAGE of expression of the pCEP4/Sec-2864933 vector in 293 cells.

FIG. 17 depicts the nucleotide (panel A) and amino acid (panel B) sequences obtained for the cDNA clone 3352358-S153A, which comprises the extracellular domain of 3352358-1, wherein the underlined sequences depict flanking sequence.

15 FIG. 18 depicts expression of the pCEP4/Sec-3352358 vector in 293 cells analyzed in a Western blot of 293 cell extracts after reducing SDS-PAGE.

Figure 19 depicts real time quantitative PCR (TaqManTM) analysis of the expression of clone 2864933 utilizing primer-probe set 88 (Panel A), primer-probe set 291 (Panel B), and primer-probe set 341 (Panel C).

20 Figure 20 depicts real time quantitative PCR (TaqManTM) analysis of the expression of 3352358.

Figure 21 depicts real time quantitative PCR (TaqManTM) analysis of the expression of 3911675.

Figure 22 depicts real time quantitative PCR (TaqManTM) analysis of the expression of 4035508.

25 Figure 23 depicts real time quantitative PCR (TaqManTM) analysis of the expression of 4339264.

DETAILED DESCRIPTION OF THE INVENTION

The present invention is based, in part, upon the discovery of novel polynucleotide sequences and the membrane-bound or secreted polypeptides encoded by these sequences. Polypeptides encoded by nucleotides of the invention include clone 2777610 (a chemokine receptor-like protein, SEQ ID NOS:1-2); assembled clones 2864933-1 and 2864933-2 and the pCEP4/Sec-2864933 vector and cDNA clone pCR2.1-2864933 (semaphorin protein-like splice variants, SEQ ID NOS:3-6, 29-30); clone 2982339 (a putative mitochondrial protein, SEQ ID NOS:7-8), assembled clones 3352358-1 and 3352358-2 and the cDNA clone 3352358-S153A (SLIT protein-like splice variants, SEQ ID NOS:9-12, 31-32); clones 3884846, 3884846 -1 and 3884846 -2 (putative microbody/peroxisome associated

protein splice variants, SEQ ID NOS:13-14 and 74-77), clones 3911675 and 3911675 -2 (tetraspanin-like protein splice variants, SEQ ID NOS:15-16 and 78-79); clones 4004056 and 4004056.0.143u (putative proline-rich membrane protein splice variants, SEQ ID NOS:17-18 and 80-81); clone 4004731-1 (a laminin β -chain precursor-like protein, SEQ ID NOS:19-20); clones 4009334-1 and 4009334-2 (AVENA protein-like splice variants, SEQ ID NOS:21-24); clone 4035508 (a novel fetal lung-associated protein, SEQ ID NOS:25-26); and clone 4339264 (a myeloid upregulated protein, SEQ ID NOS:27-28). These genes are collectively referred to as the SECX gene set. The polynucleotides and polypeptides are set forth in Table 1. Table 1 lists the SEQ ID NOs for each nucleotide and amino acid sequence of the invention, as well as SEQ ID NOs for the primers specific 10 to the clones of this invention that were employed in various aspects and embodiments described herein.

Table 1: Sequences and Corresponding SEQ ID Numbers

Clone or Primer No.	Figure	Nucleic acid SEQ ID NO:	Protein SEQ ID NO:
2777610	1	1	2
2864933-1	2	3	4
2864933-2	3	5	6
2982339	4	7	8
3352358-1	5	9	10
3352358-2	6	11	12
3884846	7A	13	14
3884846-1	7B	74	75
3884846-2	7C	76	77
3911675	8A	15	16
3911675-2	8B	78	79
4004056	9A	17	18
4004056.0.143u	9B	80	81
4004731-1	10	19	20
4009334-1	11	21	22
4009334-2	12	23	24
4035508	13	25	26
4339264	14	27	28
pCR2.1-2864933	15	29	30
3352358-S153A	17A, 17B	31	32
2864933 MatF		33	
2864933 F-TOP0-Reverse		34	
2864933-Seq-0		35	
2864933-Seq-1		36	
2864933-Seq-2		37	
2864933-Seq-3		38	
2864933-Seq-4		39	
2864933-Seq-5		40	
2864933-Seq-6		41	
pSec-V5-His Forward		42	
pSec-V5-His Reverse		43	

3352358CForward		44	
3352358CReverse		45	
3352358 Seq-1		46	
3352358 Seq-2		47	
3352358 Seq-3		48	
3352358 Seq-4		49	
Ag 111 (F)		50	
Ag 111 (R)		51	
Ag 111 (P)		52	
Ag 88 (F)		53	
Ag 88 (R)		54	
Ag 88 (P)		55	
Ag 291 (F)		56	
Ag 291 (R)		57	
Ag 291 (P)		58	
Ag 341 (F)		59	
Ag 341 (R)		60	
Ag 341 (P)		61	
Ag 42 (F)		62	
Ag 42 (R)		63	
Ag 42 (P)		64	
Ag 115 (F)		65	
Ag 115 (R)		66	
Ag 115 (P)		67	
Ag 118 (F)		68	
Ag 118 (R)		69	
Ag 118 (P)		70	
Ag 120 (F)		71	
Ag 120 (R)		72	
Ag 120 (P)		73	

1. **Clone 2777610**

Clone 2777610 is a 1812 bp nucleic acid sequence (SEQ ID NO:1) that was originally identified in bone tissue, which includes bone marrow. The full length clone (FIG. 1) was further assembled from sequences expressed in bone tissues. An open reading frame ("ORF") encoding a polypeptide (SEQ ID NO:2) having 333 amino acid residues is found at nucleotides 537 – 1535 (FIG. 1). The nucleotide sequence includes a Kozak sequence. The stop codon TGA is found at nucleotides 1536-1538. The results of a PSORT analysis predict that the protein is localized in the plasma membrane with a certainty of 0.6000. The SignalP program predicts that a signal sequence occurs with a most likely cleavage site between residues 44 and 45, represented by the dash between the amino acids TLA-LW (*i.e.*, ThrLeuAla - LeuTrp).

The protein of clone 2777610 has 332 of 333 residues both (99%) identical and positive to a human seven transmembrane receptor protein designated HNEAA81 (European Patent number 913471-A2). As used herein, "identical" residues correspond to those residues in a comparison

between two sequences where the equivalent nucleotide base or amino acid residue in an alignment of two sequences is the same residue. Residues are “positive” when the comparisons between two sequences in an alignment show that residues in an equivalent position in a comparison are either the same amino acid or a conserved amino acid as defined below. Clone 2777610 was also found to have 5 328 of 333 residues both (98%) identical and positive to a human chemokine receptor-like protein (PCT Publication WO9839441-A1). A weaker similarity was also detected for a 338 residue human probable G protein-coupled receptor KIAA0001 (GenBank Accession number Q15391).

Members of the G protein-coupled receptor (GPCR) superfamily contain seven transmembrane domains and transduce extracellular signals through heterotrimeric G proteins. G-10 protein-coupled receptors (GPCRs) are integral membrane proteins of great pharmacological importance owing to their central role in the regulation of cellular responses to external stimuli. See, for example, Marchese *et al.*, 1999 *Trends Pharmacol Sci* 20(9): 370-5; and Rozengurt 1998 *J Cell Physiol* 177(4):507-17.

15 GPCR receptors specifically bind select neurotransmitters and peptide hormones, and are likely to underlie the recognition and G-protein-mediated transduction of various signals. These signals activated by ligand-bound GPCRs have been implicated in a variety of normal and abnormal processes, including development, inflammation, and malignant transformation (matrix invasion, motility, chemotaxis, adhesion, growth and survival signaling). These signaling peptides exert their characteristic effects on cellular processes by binding to specific GPCRs on the surface of their target 20 cells. Typically, the binding of a neuropeptide to its cognate GPCR triggers the activation of multiple signal transduction pathways that act in a synergistic and combinatorial fashion to relay the mitogenic signal to the nucleus and promote cell proliferation. A rapid increase in the synthesis of lipid-derived second messengers with subsequent activation of protein phosphorylation cascades is an important early response to neuropeptides. An emerging theme in signal transduction is that these agonists also 25 induce rapid and coordinate tyrosine phosphorylation of cellular proteins including the nonreceptor tyrosine kinase p125fak and the adaptor proteins p130cas and paxillin. This tyrosine phosphorylation pathway depends on the integrity of the actin cytoskeleton and requires functional Rho.

The Clone 2777610 protein is a seven transmembrane receptor protein with chemokine receptor-like properties. Clone 2777610 is a G protein coupled receptor (GPCR) that is a likely 30 gamma-aminobutyric acid receptor, in the class of P2Y-like GPCRs. As such, Clone 2777610 is useful in diagnosing and/or treating pathologies and disorders associated with G-protein coupled receptor metabolism, *e.g.*, bacterial disease; asthma; fungal disease; viral disease; HIV-1; HIV-2; cancer; anorexia; Parkinson's disease; hypertension; osteoporosis; myocardial infarction; manic depression; schizophrenia; Gilles de la Tourette's syndrome; inflammatory disorder; and viral infection.

35 Based on the roles of other GPCRs and the high expression of 2777610 in lymphoid tissue such as the spleen, bone marrow, lymph node (see Example 6), the inventor anticipates that successful

therapeutic targeting of 2777610, using either small molecules that inhibit transmembrane signaling by 2777610 or monoclonal antibodies designed to block the interaction of 2777610 with ligand(s), might have utility in modulating lymphoproliferative disorders (myeloma, myeloid leukemia, non-Hodgkin's lymphoma, etc.) or autoimmune diseases (SLE, etc.) or both. Likewise, with respect to the 5 high expression of 2777610 in whole adult human brain, hippocampus, substantia nigra and spinal cord, modulation of 2777610 signaling using the approaches described above will have clinical utility to modulate certain neurodegenerative disorders affecting motor function.

2. Clone 2864933-1

Clone 2864933-1 includes a nucleic acid sequence (SEQ ID NO:3) including 3498 nucleotides (FIG. 2). This clone is similar to clone 2864933-2 (below) with the exception that 2864933-1 has an insert of 164 nucleotides at positions 1942-2106. The gene fragment giving rise to this clone was found mainly in heart tissue. Fragments included in this gene were also found in lymph node, pancreas, thalamus, brain, salivary gland and adrenal gland. Clone 2864933-1 includes a Kozak sequence, a start codon at nucleotides 214-216, and a TAA termination codon at nucleotides 3031-15 3033. The nucleotide residues between 214-3030 define an ORF encoding a protein (SEQ ID NO:4) of 939 amino acid residues (FIG. 2). Molecular cloning and expression of a fragment corresponding to the putative mature extracellular domain of 2864933-1 is given in Examples 2 and 3. The PSORT program predicts that the 2864933-1 protein localizes to the plasma membrane with a certainty of 0.4600. The protein is a likely Type I transmembrane protein, with the predicted transmembrane 20 domain between residues 645-661 of SEQ ID NO:4. The SignalP program predicts that the protein has a signal peptide cleavage site between residues 18 and 19, represented by the dash between the amino acids AGA-GF (*i.e.*, AlaGlyAla - Gly Phe).

The 2864933-1 protein is 94% identical, and 97% similar, to a murine semaphorin polypeptide having 888 amino acid residues (GenBank Accession Number AAB86408). In addition, it shows 35% 25 identity and 53% similarity to human semaphorin III (GenBank Accession Number AAA65938). For these reasons the 2864933-1 polypeptide is believed to be a cytokine-like growth factor.

The semaphorin (a.k.a. collapsin) family of molecules plays a critical role in the guidance of nerve growth cones during neuronal development. The family is characterized by the presence of a conserved semaphorin domain at the amino terminus. Mutational analysis of human semaphorin A(V) 30 revealed mutations (germline in 1 case) in 3 of 40 lung cancers. Semaphorin E is responsible for a non-MDR drug resistance in human cancers including ovarian cancer and is overexpressed in CDDP-resistant cell lines as well as induced by diverse chemotherapeutic drugs and by X-ray and UV irradiation. Yamada *et al.* 1997 *Proc. Nat. Acad. Sci.* 94: 14713-14718. Human semaphorin E mRNA is up-regulated in synovial fibroblasts of rheumatoid arthritis patients. Mangasser-Stephan *et al.* 1997 35 *Biochem. Biophys. Res. Commun.* 234: 153-156. Human neuropilin-1, a receptor for the

collapsin/semaphorin family that mediates neuronal cell guidance, is expressed by endothelial and tumor cells as an isoform-specific receptor for vascular endothelial growth factor and is believed to regulate VEGF-induced angiogenesis. Soker *et al.* 1998 *Cell* 92: 735-745.

Semaphorins, the plexin family of semaphorin receptors, and scatter factor receptors share evolutionarily conserved protein modules, *e.g.*, the semaphorin domain and Met Related Sequences (MRS). Artigiani *et al.*, 1999, *IUBMB Life* 48(5):477-82. These proteins have a common role of mediating cell guidance cues. During development, scatter factor receptors control cell migration, epithelial tubulogenesis, and neurite extension. Semaphorins and their receptors are known signals for axon guidance. They are also believed to regulate developmental processes involving cell migration and morphogenesis, and have been implicated in immune function and tumor progression. Scatter factors and secreted semaphorins are diffusible ligands, whereas membrane-bound semaphorins signal by cell-cell interaction. Cell guidance control by semaphorins requires plexins, alone or in a receptor complex with neurophilins. Semaphorins, besides their role in axon guidance, are expected to have multiple functions in morphogenesis and tissue remodeling by mediating cell-repelling cues through plexin receptors.

The potential roles of the 2864933 protein in tumorigenesis include development of chemoresistance, radiotherapy resistance, survival in trophic factor limited secondary tissue site microenvironments, potential involvement in enhancing VEGF-induced angiogenesis

Based on the reported roles of semaphorins summarized herein it is anticipated that successful therapeutic targeting of 2864933 and/or its splice variants will result in significant anti-tumor activity in combination with established cytotoxic/genotoxic therapies (*i.e.* chemosensitization, radiosensitization). Additionally, the semaphorins play roles in axon outgrowth and neuronal cell migration. In this regard successful therapeutic targeting of 2864933-1 and/or 2864933-2 might also limit the extent (frequency) of metastatic dissemination (tumor burden) and potentially limit tumor angiogenesis. Therapeutic targeting of 2864933 and its splice variants is also provided via the generation of human or humanized monoclonal antibodies that block the ability of 2864933-1 or 2864933-2 to interact with cognate ligand(s) and elicit a transmembrane signal(s). Equally, the generation of small molecules (synthetics, cell permeable peptides, other) than specifically interfere with one or more of the downstream signaling components in the pathway(s) activated by ligand-bound 2864933-1 and/or 2864933-2 would be expected to have significant anti-tumor activity as described above. Likewise, the introduction of antisense constructs (naked DNA, adenoviral constructs), ribozymes to inhibit the expression of 2864933-1 and/or 2864933-1 would be expected to have significant anti-tumor activity as described above.

Based on the expression profiles of the 2864933-1 and 2864933-2 transcripts set forth in Example 7 and FIGS. 19A, 19B and 19C, therapeutic indications for targeting 2864933-1 and 2864933-2 include renal cell carcinomas, small cell lung cancers, large cell variants of small cell lung

cancer, breast adenocarcinomas, and malignant melanomas. Clones 2864933-1 and 2864933-2 are useful in diagnosing and/or treating pathologies related to developmental malfunction, especially in the nervous system, and in treatment of CNS pathologies, e.g., Alzheimer's disease and parkinsonism.

3. **Clone 2864933-2**

5 Clone 2864933-2 has a nucleic acid sequence (SEQ ID NO:5) of 3333 nucleotides (FIG. 3). This clone is similar to clone 2864933-1 (above; FIG. 2) with the exception that 2864933-1 has an insert of 164 nucleotides at positions 1942-2106(according to the SEQ ID NO:3 numbering for clone 2864933-1). This difference appears to be an RNA splicing variation. The gene fragment giving rise to this clone 2864933-2 was found mainly in heart tissue. Transcribed sequences from this gene are also 10 found in lymph node, pancreas, thalamus, brain, salivary gland and adrenal gland. Clone 2864933-1 includes a Kozak sequence, a start codon at nucleotides 214-216, and a TAA termination codon at nucleotides 2866-2868. The nucleotides between 214 and 2865 thus define an ORF encoding a protein (SEQ ID NO:6) of 884 amino acid residues (FIG. 3). The PSORT program predicts that the 2864933-1 protein localizes to the plasma membrane with a certainty of 0.4600. The SignalP program predicts 15 that the protein most likely has a signal peptide cleavage site between residues 18 and 19, represented by the dash between the amino acids AGA-GF (*i.e.*, AlaGlyAla - GlyPhe).

The 2864933-2 protein is 95% identical, and 97% similar to murine semaphorin having 888 amino acid residues (GenBank Accession number AAB86408). In addition, it shows 38% identity and 55% similarity to human semaphorin III (GenBank Accession number AAA65938).

20 The 2864933-2 protein was also found to have 869 of 877 residues (99%) identical, and 871 of 877 residues (99%) positive, to the 974 residue human secreted protein from a clone designated CJ145-1 (PCT Publication WO9827205-A2). The 2864933-2 sequence was isolated from a human fetal brain cDNA library and is a novel secreted protein distinct from clone CJ145-1 in both size and sequence. Clone 2864933-2 is useful for cytokine and cell proliferation/differentiation activity, 25 immune stimulating or suppressing activity, hematopoiesis regulating activity, tissue growth activity, activin/inhibin activity, chemotactic/chemokinetic activity, hemostatic and thrombotic activity, receptor/ligand activity, anti-inflammatory activity, cadherin/tumor invasion suppressor activity, tumor inhibition activity and other activities. The Clone 2864933-2 is also useful in diagnosing and/or treating pathologies related to developmental malfunction, especially in the nervous system, and in the 30 treatment of CNS pathologies, e.g., Alzheimer's disease and parkinsonism.

4. **Clone 2982339**

Clone 2982339 has a sequence (SEQ ID NO:7) of 856 nucleotides (FIG. 4), including a Kozak sequence, an initiation codon at positions 138-140 and a TGA stop codon at positions 726-728. This sequence between residues 138 to 725 defines an open reading frame encoding a protein (SEQ ID 35 NO:8) of 196 amino acid residues (FIG. 4). The clone originated from fetal brain and was assembled

using 65 sequences from fetal thymus and placenta. Fragments for this clone are also found in human placenta, thymus gland, thyroid gland, and bone, including osteosarcomas. The PSORT predicts that the 2982339 protein localizes to the mitochondrial matrix space with a certainty of 0.7077. SignalP suggests that the protein may have no known N-terminal signal sequence.

5 The 2982339 protein has 16 of 54 residues (29 %) identical to, and 24 of 54 residues (44%) positive with, an artificial sequence of 109 residues that is an aprotinin analogue precursor (GenBank Accession numbers AAB54954 and AAB54956). Aprotinin, also known as pancreatic trypsin inhibitor precursor or basic protease inhibitor, is an intracellular polypeptide found in many tissues, and is a known inhibitor of trypsin, kallikrein, chymotrypsin, and plasmin. GenBank Accession number
10 P00974; Creighton and Charles, 1987 *J. Mol. Biol.* 194 (1): 11-22.

5. **Clone 3352358-1**

Clone 3352358-1 includes a 2341 nucleotide sequence (SEQ ID NO:9) (FIG. 5) with an initiation codon at nucleotides 215-217 and a TAA stop codon at nucleotides 2174-2176. This sequence between residues 215 to 2173 defines an ORF encoding a protein (SEQ ID NO:10) of 653 residues (FIG. 5). The clone was identified by a polynucleotide fragment originating in fetal liver. Expressed sequences are also found in liver, including fetal liver, kidney, including fetal kidney, and thalamus. The PSORT program predicts that the 3352358-1 protein localizes in the plasma membrane with a certainty of 0.46. The SignalP program predicts that the protein has a signal peptide, with the most likely cleavage site between residues 38 and 39, represented by the dash between the amino acids
20 AAA-AS (*i.e.*, AlaAlaAla - AlaSer), or between residues 41 and 42, represented by the dash between the amino acids ASA-GP (*i.e.*, AlaSerAla - GlyPro). The protein is predicted to be a Type I transmembrane protein with the transmembrane domain located between residues 522 and 551.

The 3352358-1 protein has 35% of its residues identical, and 48% of its residues similar to, human slit-1 protein, a protein of 1534 residues (GenBank Accession number BAA35184). 3352358-1 protein is also 39% identical and 46% similar to human slit-3 protein, a protein of 1523 residues (GenBank Accession number BAA35186); and 40% identical and 48% similar to the human neurogenic extracellular slit protein slit-2 having 1521 residues (GenBank Accession number AAD04309). The 3352358-1 protein has an overall 53% identity to a hypothetical 45.1 kDa protein (GenBank Accession number CAB70473).

30 The *slit* genes encode proteins with a conserved chemorepulsive activity for axons in invertebrates and vertebrates. Chen *et al.*, 2000, *Neuroscience* 96: 231-236; Yuan *et al.*, 1999 *Dev Biol* 212: 290-306. For example, the binding of Slit to Roundabout, expressed on the cell surface, is implicated in neuronal guidance activity. Thus, Slit proteins may guide axon projections in multiple regions of the embryo.

By analogy, Clone 3352358-1 has diagnostic and therapeutic utility in pathologies related to neural development and in CNS pathologies, e.g., Alzheimer's disease and parkinsonism.

Molecular cloning and expression of the putative mature extracellular domain of 3352358-1 is described in Examples 4 and 5. This cloned fragment originated from cDNA samples obtained from 5 human testis and fetal brain. The resulting clone, designated clone 3352358-S153A, differs in sequence from that shown in FIG. 5. The respective 3352358-S153A nucleotide sequence is disclosed in FIG. 17A (SEQ ID NO:31) and polypeptide sequence is disclosed in FIG 17B (SEQ ID NO: 32). One reason for the sequence difference between the 3352358-1 clone and 3352358-S153A cDNA is likely the tissue or organ sources of the cDNAs. If so, this finding represents a tissue-specific or 10 organ-specific basis for allelic variants (also known as isoforms) of proteins, e.g., the disclosed 3352358-1 slit-like protein. The 3352358-1 and 3352358-S153A clones will thus have utility in identifying those tissue or cell types that express these allelic or splice variants.

The 3352358 sequence is related to MEGF (multiple epidermal growth factor-like domains)/Slit family and roundabout. The domain that characterizes epidermal growth factor consists 15 of approximately 50 amino acids with 3 disulfide bonds. EGF-like domains are believed to play a critical role in a number of extracellular events, including cell adhesion and receptor-ligand interactions. Proteins with EGF-like domains often consist of more than 1,000 amino acids, have multiple copies of the EGF-like domain, and contain additional domains known to be involved in specific protein-protein interactions.

20 Important members of this family include fat tumor suppressor, (*Drosophila*, homolog of, 2; fat2). The *Drosophila* fat gene is a tumor suppressor gene whose product controls cell proliferation and morphogenesis in the imaginal discs in a contact-dependent manner. Another relative of 3352358 is Slit1 (also known as MEGF4), a *Drosophila* gene involved in the formation and maintenance of the nervous and endocrine systems. Another relative of 3352358 is roundabout, a *Drosophila* gene that 25 controls axon crossing of the CNS midline and defines a novel subfamily of evolutionarily conserved guidance receptors. Kidd *et al.* 1998 *Cell* 92: 205-215; Nakayama *et al.* 1998 *Genomics* 51: 27-34.

The potential role(s) of 3352358 in Tumorigenesis include chemoresistance, radiotherapy resistance, survival in trophic factor limited secondary tissue site microenvironments, potential involvement in angiogenesis.

30 Based on the reported roles of MEGFs/SLITs/Roundabout described herein it is anticipated that successful therapeutic targeting of 3352358 and/or its splice variants will result in significant anti-tumor activity in combination with established cytotoxic/genotoxic therapies (i.e. chemosensitization, radiosensitization). Additionally, the semaphorins play roles in axon outgrowth and neuronal cell migration. In this regard successful therapeutic targeting of 3352358 might also limit the extent 35 (frequency) of metastatic dissemination (tumor burden) and potentially limit tumor angiogenesis. Therapeutic targeting of 3352358 and its splice variants will also be provided via the generation of

human or humanized monoclonal antibodies that block the ability of 3352358 to interact with cognate ligand(s) and elicit a transmembrane signal(s). Equally, the generation of small molecules (synthetics, cell permeable peptides, other) than specifically interfere with one or more of the downstream signaling components in the pathway(s) activated by ligand-bound 3352358 will have significant anti-tumor activity as described above. Likewise, the introduction of antisense constructs (naked DNA, adenoviral constructs), ribozymes to inhibit the expression of 3352358 will have significant anti-tumor activity as described above.

Based on the expression profiles of the 3352358 transcripts presented in Example 8 and FIG. 20, the therapeutic indications for targeting 3352358 include select hepatomas/hepatocellular carcinomas and renal cell carcinomas.

6. **Clone 3352358-2**

Clone 3352358-2 of 2607 nucleotides (SEQ ID NO:11) includes a Kozak sequence, an initiation codon at nucleotides 215-217 and a TAA termination codon at nucleotides 1985-1987 (FIG. 6). This sequence between residues 215 to 1984 defines an ORF encoding a protein (SEQ ID NO:12) of 590 residues (FIG. 6). The PSORT program predicts that the 3352358-2 localizes in the plasma membrane. The SignalP program predicts that the protein has a signal peptide, with the most likely cleavage site between residues 38 and 39, represented by the dash between the amino acids AAA-AS (*i.e.*, AlaAlaAla - AlaSer). This clone originates in human liver, including adult and fetal liver. Transcribed sequences from this gene are found in liver, including fetal liver, bone, including bone marrow, brain, and the pituitary gland.

Similarity searches indicate that the 3352358-2 protein is 35% identical and 48% similar with human slit-1 protein (GenBank Accession number BAA35184) having 1534 residues. The slit genes encode proteins with a conserved chemorepulsive activity that affects axons from both invertebrates and vertebrates. Chen *et al.*, 2000, *Neuroscience* 96(1):231-236; Yuan *et al.*, 1999 *Dev Biol* 212(2):290-306. Binding of Slit to the Roundabout protein expressed on a cell surface is implicated in this neuronal guidance activity. Thus Slit proteins guide axon projections in multiple regions of the developing embryo.

Clone 3352358-2 will have diagnostic and therapeutic utility in pathologies related to neural development and CNS pathologies, *e.g.*, Alzheimer's disease and parkinsonism.

30 7. **Clone 3884846**

Clone 3884846 includes a polynucleotide (SEQ ID NO:13) of 1340 nucleotides (FIG. 7A) having a Kozak sequence, an initiation codon at nucleotides 421-423 and a TAG stop codon at nucleotides 1288-1290. This sequence between residues 421 through 1287 defines an ORF encoding a protein (SEQ ID NO:14) of 289 amino acid residues (FIG. 7A). Transcribed sequences from this gene are found in pituitary gland, testis, kidney, including fetal kidney, brain, including fetal brain, pituitary

gland, placenta, pancreas, testis, spleen kidney, including fetal kidney, fetal liver, skeletal muscle, heart, OVCAR-3 cells and lung. The PSORT program predicts that the 3884846 protein localizes to the microbody (peroxisome) with a certainty of 0.7480. There appears to be no known signal peptide in the protein.

5 Clone 3884846-1 does not have an initiation codon at the beginning of the nucleotide sequence (SEQ ID NO:74), so it is believed that the disclosed clone represents an incomplete ORF. It is assumed that an initiation codon is found upstream from the sequence shown, such that the cDNA sequence extends further 5' from that shown in FIG. 7B. A stop codon of TGA is located at nucleotides 979-981. The 3884846-1 nucleotide sequence between residues 1 to 978 defines an ORF 10 encoding a protein (SEQ ID NO:75) of 326 amino acid residues (FIG. 7B).

A full length Clone 3884846-2 includes the nucleic acid sequence (SEQ ID NO:76) shown in Fig. 7C. Clone 3884846-2 has an initiation codon at nucleotides 299-301 and a TGA stop codon at nucleotides 983-985. This sequence between residues 299 to 982 defines an ORF encoding a protein (SEQ ID NO:77) of 228 amino acid residues (FIG. 7C)

15 8. **Clone 3911675**

Clone 3911675 is a polynucleotide (SEQ ID NO:15) of 1428 nucleotides (FIG. 8A). The nucleotide sequence includes a Kozak sequence, a start codon at positions 96-98, and a TGA stop codon at nucleotides 906-908. This sequence between residues 96 through 905 define an ORF 20 encoding a protein (SEQ ID NO:16) having 270 amino acid residues (FIG. 8A). The clone originates in DNA isolated from spleen cells. The PSORT program predicts that the protein is localized in the plasma membrane. According to the SignalP program, the protein is predicted to have a signal peptide with the most probable cleavage site between 42 and 43, represented by the dash between the amino acids AWS-EK (*i.e.*, AlaTrpSer - GluLys).

Clone 3911675-2 does not have an initiation codon at the beginning of the nucleotide 25 sequence (SEQ ID NO:78), so it is believed that the disclosed clone represents an incomplete ORF. It is assumed that an initiation codon is found upstream from the sequence shown, such that the cDNA sequence extends further 5' from that shown in FIG. 8B. A stop codon of TGA is located at nucleotides 629-631. The 3911675-2 nucleotide sequence between residues 2 to 628 defines an ORF encoding a 3911675-2 protein (SEQ ID NO:79) of 209 amino acid residues (FIG. 8B).

30 In database searches for similarity, the 3911675 protein is 57% identical to, and 75% positive with, human tetraspan NET-4 protein of 268 residues (GenBank Accession Number AAC17120), and is 57% identical to, and 74% positive with, human tetraspanin TSPAN-5 having 264 residues (GenBank Accession Number NP005714).

35 TM4SF4 (transmembrane 4 superfamily member 4), is an integral membrane glycoprotein found to regulate the adhesive and proliferative status of intestinal epithelial cells through a density-

dependent mechanism. Members of the 'transmembrane 4 superfamily' (TM4SF) are cell-surface proteins presumed to have 4 transmembrane domains. Many tetraspan proteins are considered "promiscuous" interactors by virtue of their associations with other molecules, including lineage-specific proteins, integrins, and other tetraspanins. Tetraspan proteins are involved in diverse processes, e.g., cell activation and proliferation, adhesion and motility, differentiation, and cancer.

Maecker *et al.* 1997 *FASEB J* 11(6): 428-42. The tetraspan family proteins function as "molecular facilitators, grouping specific cell-surface proteins and thus increasing the formation and stability of functional signaling complexes" and so aid in the formation of plasma membrane signaling complexes. Maecker *et al.* 1997 *FASEB J* 11(6): 428-42; Birling *et al.*, 1999 *J. Neurochem* 73(6): 2600-2008. Neuronal tetraspanin family members are implicated in axon growth and target recognition. Perron and Bixby 1999 *FEBS Lett* 461(1-2): 86-90.

Based on the reported roles of tetraspan-related proteins described herein it is anticipated that successful therapeutic targeting of 3911675 and/or its splice variants will result in significant anti-tumor activity (tumor growth inhibition) especially in combination with established cytotoxic/genotoxic therapies (i.e. chemosensitization, radiosensitization). In this regard successful therapeutic targeting of 3911675 might also limit the extent (frequency) of metastatic dissemination (tumor burden) and potentially limit tumor angiogenesis. Therapeutic targeting of 3911675 and its splice variants will also be provided via the generation of human or humanized monoclonal antibodies that block the ability of 3911675 to interact with specific cognate ligand(s) and elicit a transmembrane signal(s). Equally, the generation of small molecules (e.g., synthetics, cell permeable peptides) that specifically interfere with one or more of the downstream signaling components in the pathway(s) activated by ligand-bound 3911675 will have significant anti-tumor activity as described above. Likewise, the introduction of antisense constructs (naked DNA, adenoviral constructs), ribozymes to inhibit the expression of 3911675 will have significant anti-tumor activity as described above.

Clone 3911675 will thus have diagnostic and therapeutic utility in pathologies related to cell signaling and neural development and in CNS pathologies, e.g., Alzheimer's disease and parkinsonism. Based on the ubiquitous expression profiles of the 3911675 gene (see Example 9 and FIG. 21), one specific therapeutic indication for targeting 3911675 is for malignant melanoma.

9. **Clone 4004056**

Clone 4004056 includes a nucleic acid sequence (SEQ ID NO:17) of 1767 nucleotides (FIG. 9). There is an initiation codon at positions 51-53 and a TAA stop codon at positions 984-986. Nucleotides from 51 to 983 therefore define an ORF encoding a protein (SEQ ID NO:18) of 311 amino acid residues (FIG. 9). The clone was originally identified in salivary gland. Transcribed sequences from this gene are found in total RNA libraries from adrenal gland, placenta, mammary tissue, prostate, testis, uterus, spleen, fetal thymus (CRL7046), osteogenic sarcoma cells (HTB96),

fetal lung, thalamus, fetal kidney and Burkitt's lymphoma (*i.e.*, Raji cells), and in mRNA libraries from bone marrow, melanoma, pituitary, thyroid. The PSORT program predicts that the 4004056 protein is localized in the plasma membrane. SignalP predicts no known signal peptide for this protein, however.

5 Fig. 9B shows the nucleotide sequence (SEQ ID NO:80) and translated protein sequence (SEQ ID NO:81) for clone 4004056.0.143u. Clone 4004056.0.143u has an initiation codon at nucleotides 63-65 and a TGA stop codon at nucleotides 1023-1025. This sequence between residues 63 and 1022 defines an ORF encoding a protein (SEQ ID NO:81) of 320 amino acid residues (FIG. 9B)

10 Database searches indicate that the 4004056 protein has 306 of 311 residues (98%) both identical to and positive with a 311 residue human transmembrane domain containing protein from clone HP01862, thought to control cell proliferation and differentiation. (PCT Publication WO9927094-A2). Similarly, the protein has 306 of 311 residues (98%) identical to and positive with a 311 residue human protein (SEQ ID NO:10 from PCT Publication WO9927094-A2). Clone 4004056 furthermore has 305 of 311 residues (98%) both identical to and positive with the human 311 residue 15 proline-rich membrane protein (PCT Publication WO9833910-A1). In other searches it was found that the 4004056 protein has 153 of 284 residues identical to (53%), and 196 of 284 residues positive with (69%), the 316 residue rat neural membrane protein 35 (GenBank Accession Number AAC32463). In addition, the protein is 42% identical to, and 65% positive with, a 208 residue fragment of human NMDA receptor glutamate-binding chain (GenBank Accession Number AAB94292).

20 The novel 4004056 clone has a range of activities including cytokine and cell differentiation, immune stimulation/suppression, hematopoiesis regulation, tissue growth, activin/inhibin activities, chemostatic/chemokinetic activities, hemostatic/thrombolytic activities, receptor/ligand activities, tumor inhibitor, anti-inflammatory and additional undefined activities. The 4004056 cDNAs has utility as a probes for gene diagnosis and as gene sources for gene therapy. These cDNAs are also 25 useful for large scale expression of proteins. Cells transformed with various 4004056 nucleotides are useful for detection of the corresponding ligands and for screening of novel low-molecular weight pharmaceuticals.

30 The 4004056 protein is a likely human proline-rich membrane protein (PRMP). PRMP is similar to rat NMDA receptor glutamic acid binding subunit. PRMP is involved in cell signaling, protein trafficking and subcellular localization, control of cell architecture, cell-cell interactions, cell growth and development, and modulation of immune and inflammatory responses. The PRMP and agonists can be used to promote tissue or organ regeneration. The antagonists or inhibitors of PRMP is useful for treating or preventing disorders associated with expression of PRMP, *e.g.* inflammatory and allergic conditions such as rheumatoid and osteoarthritis, asthma, allergic rhinitis, atopic dermatitis, 35 autoimmune conditions such as Sjogren's syndrome, scleroderma, hyperthyroidism (Grave's disease), systemic lupus, myasthenia gravis, autoimmune thyroiditis, diabetes mellitus, pancreatitis, ulcerative

colitis, Crohn's disease, atrophic gastritis, and graft versus host disease, disorders relating to abnormal cellular differentiation, proliferation, or degeneration, including arteriosclerosis, atherosclerosis, hyperaldosteronism, hypocortisolism (Addison's disease), hypothyroidism, colorectal polyps, gastric and duodenal ulcers, cancers of hematopoietic cells and lymphoid tissues including leukemias, 5 lymphomas (including Hodgkin's disease), lymphosarcomas and myelomas, and carcinomas of glands, tissues, and organs involved in secretion or absorption, and organs of the gastrointestinal tract.

10. Clone 4004731-1

Clone 4004731-1 is a polynucleotide (SEQ ID NO:19) comprising 1686 nucleotides (FIG. 10). The clone has a Kozak sequence, an initiation codon at positions 372-374 and a TAA termination 10 codon at nucleotides 1278-1280. The nucleotide residues between 372 and 1277 thus define an ORF encoding a protein (SEQ ID NO:20) having 302 amino acid residues (FIG. 10). The PSORT predicts that the protein localizes to the mitochondrial matrix space with a low certainty of 0.3600. The program SignalP predicts that no known signal peptide is present. Transcribed sequences from this gene are found in brain, pituitary, heart, breast and spleen.

15 In similarity searches it was found that the 4004731-1 protein has 50% identity and 67% similarity with the human laminin beta-1 chain precursor (laminin B1 chain), a protein having 1786 residues (GenBank Accession Number P07942). Laminins are a major component of the basement membrane and have several biologically active sites that regulate angiogenesis and tumor growth. Grant *et al.*, 1994 *Pathol Res Pract.* 190(9-10): 854-863. Laminins strongly stimulate axon outgrowth 20 *in vitro*, and are transiently expressed in embryonic development and after CNS injury. Luebke *et al.*, 1995 *J. Neurobiol* 27(1): 1-14. In addition, Laminin B1 expression is greatly disturbed in severely diseased patients with severe childhood autosomal recessive muscular dystrophy. Yamada *et al.*, 1995 *Lab Invest.* 72(6): 715-722. Clone 4004731-1 thus has diagnostic and therapeutic utility in pathologies related to muscular dystrophy, cell outgrowth, cell proliferation, angiogenesis, and neural 25 development and in CNS pathologies, e.g., CNS injury, Alzheimer's disease and parkinsonism.

11. Clone 4009334-1

Clone 4009334-1 includes a polynucleotide (SEQ ID NO:21) having 2010 nucleotides (FIG. 11). This clone is similar to clone 4009334-2 (below), but is longer than the latter because of inserts at 11 nucleotides 1361-1440 and 1541-1597 in SEQ ID NO:21. These differences are thought to arise from 30 splicing variations of the mRNA. Clone 4009334-1 has a start codon at positions 243-245 and a TGA termination codon at nucleotides 1659-1661. Residues between 243 and 1658 therefore define an ORF encoding a protein (SEQ ID NO:22) of 472 residues (FIG. 11). The PSORT software program affords a weak prediction that the 4009334-1 protein localizes to the microbody (certainty = 0.30). The SignalP software program predicts that the protein lacks a known signal peptide. Transcribed 35 sequences from this gene are found in OVCAR-3 cells, MCF-7 cells, mammary gland, lung, including

fetal lung, brain, including thalamus, adrenal gland, salivary gland, pancreas, heart, white blood cells and Raji cells.

- The 4009334-1 protein has 272 of 304 residues (89%) identical to, and 278 of 304 residues (91%) positive with, the 550 residue AVENA protein from *Gallus gallus* (chicken) (GenBank Accession Numbers AB017437 and BAA33016). It also has 218 of 251 residues (86%) identical to, and 228 of 251 residues (90%) positive with, a murine 783 residue enabled homolog (neural variant MENA+ protein) (GenBank Accession Number AAC52864). The 4009334-1 protein additionally has 94 of 146 residues (64%) identical to, and 107 of 146 residues (73%) positive with, the 380 residue human vasodilator-stimulated phosphoprotein (VASP) (GenBank Accession Number P50552).
- Clone 4009334-1 thus has diagnostic and therapeutic utility in pathologies related to cellular control, cell proliferation, cell development and cell differentiation. Clone 4009334-1 is also useful in angiogenesis, carcinogenesis, and in body and organ homeostasis.

12. Clone 4009334-2

- Clone 4009334-2 includes a polynucleotide (SEQ ID NO:23) of 1952 nucleotides (FIG. 12) that appears to be a shorter splice variant of clone 4009334-1 (SEQ ID NO:21, above). The 4009334-2 nucleic acid sequence includes a Kozak sequence, an initiation codon at nucleotides 243-245 and a stop codon at positions 1716-1718. Residues between 243 and 1715 define an ORF encoding a protein (SEQ ID NO:24) having 491 amino acid residues (FIG. 12). The PSORT program affords a weak prediction that the 4009334-1 protein localizes to the microbody (certainty = 0.30). The SignalP program predicts that the protein lacks a signal peptide. Transcribed sequences from this gene are found in OVCAR-3 cells, MCF-7 cells, mammary gland, lung, including fetal lung, brain, including thalamus, adrenal gland, salivary gland, pancreas, heart, white blood cells and Raji cells.

- The 4009334-2 protein has 272 of 304 residues (89%) identical to, and 278 of 304 residues (91%) positive with, the 550 residue d1033982 (GenBank Accession Number AB017437) AVENA protein from *Gallus gallus* (chicken) (GenBank Accession Number BAA33016). It also has 218 of 251 residues (86%) identical to, and 228 of 251 residues (90%) positive with, a murine 783 residue enabled homolog (neural variant MENA+ protein) (GenBank Accession Number AAC52864). The 4009334-2 protein additionally has 94 of 146 residues (64%) identical to, and 107 of 146 residues (73%) positive with, the 380 residue human vasodilator-stimulated phosphoprotein (VASP) (GenBank Accession Number P50552).

Clone 4009334-2 thus has diagnostic and therapeutic utility in pathologies related to cellular control, cell proliferation, cell development and cell differentiation. Clone 4009334-2 is also useful in angiogenesis, carcinogenesis, pathologies related to neoplasia, and body and organ homeostasis.

13. Clone 4035508

Clone 4035508 includes a polynucleotide sequence (SEQ ID NO:25) of 827 nucleotides (FIG. 13). The clone includes a Kozak sequence, a start codon at positions 233-235 and a TGA stop codon at nucleotides 602-604, thus setting forth an ORF between residues 233 and 601 encoding a 5 polypeptide(SEQ ID NO:26) having 123 residues (FIG. 13). The PSORT program predicts that the 4035508 protein localizes to the plasma membrane. The SignalP program predicts that the 4035508 polypeptide has a signal peptide whose most probable cleavage site occurs between residues 29 and 30, represented by the dash between the amino acids LFG-WP (*i.e.*, LeuPheGly - TrpPro). Transcribed sequences from this gene are found in fetal lung tissue, and in multiple adult tissue types, including 10 lymph node tissues.

Similarity searching reveals that the 4035508 protein has 37 of 108 residues (34%) identical to, and 55 of 108 residues (50%) positive with, a 559 residue human protein PB39 (POV1; GenBank Accession Number AAC33004), a predicted secreted protein upregulated and alternatively spliced in prostate cancer. Cole *et al.*, 1998 *Genomics* 51(2): 282-287.

15 PB39 plays a role in the development of human prostate cancer. By analogy, successful therapeutic targeting of 4035508 and/or its splice variants to a mammalian subject will result in provide significant anti-tumor activity, especially in combination with established cytotoxic/genotoxic therapies (*i.e.* chemosensitization and radiosensitization). Moreover successful therapeutic targeting of 4035508 will also limit the extent, frequency, or both of metastatic dissemination (tumor burden).
20 4035508 will also limit tumor angiogenesis since 4035508 is highly expressed in activated endothelial cells, such as human umbilical vein endothelial cells (HUVECs).

Therapeutic targeting of 4035508 and its splice variants will also be provided via generation 25 of human or humanized monoclonal antibodies that block the ability of 4035508 to interact with cognate receptor(s) and elicit a transmembrane signal(s). Equally, the generation of small molecules (synthetics, cell permeable peptides, other) than specifically interfere with one or more of the downstream signaling components in the pathway(s) activated by 4035508 bound to cognate receptor(s) will have significant anti-tumor activity as described above. Likewise, the introduction of antisense constructs (naked DNA, adenoviral constructs), ribozymes to inhibit the expression of 4035508 in select human malignancies will have significant anti-tumor activity as described above.

30 Clone 4035508 has diagnostic and therapeutic utility in pathologies related to neoplasias, cell proliferation and cellular control. Based on the expression profile of the 4035508 gene (see Example 10 and FIG. 22) the therapeutic indications for targeting 4035508 include metastatic colon carcinomas (up regulation in SW620 metastatic variant of SW480), breast adenocarcinomas, glioma/astrocytomas, small cell lung cancers and malignant melanomas.

14. Clone 4339264

Clone 4339264 includes a polynucleotide (SEQ ID NO:27) of 1063 nucleotides (FIG. 14). The clone includes an initiation codon at positions 48-50 and TAA termination codon at positions 945-947. This clone includes an ORF from residues 48 to 944 encoding a protein (SEQ ID NO:28) of 299 amino acid residues (FIG. 14). The PSORT program predicts that the 4339264 protein localizes in the plasma membrane with a certainty of 0.6000. The SignalP program predicts that there is a signal peptide whose cleavage site most likely occurs between residues 69 and 70, represented by the dash between the amino acids LQA-RF (*i.e.*, LeuGlnAla - ArgPhe). The clone originates in DNA isolated from lymph node. Transcribed sequences from this gene are found in MCF-7 cells, OVCAR-3 cells, heart, prostate, uterus, mammary gland, salivary gland, thalamus, bone marrow, lymph node, spleen, fetal liver, fetal thymus - CRL7046, and 10 human total RNA libraries from Clontech, Inc. (Palo Alto, CA; brain, fetal brain, liver, fetal liver, skeletal muscle, pancreas, kidney, heart, lung and placenta).

In a similarity search, it was found that the 4339264 protein has 194 of 219 residues (88%) identical to, and 207 of 219 residues (94%) positive with the 296 residue myeloid upregulated protein of mouse (GenBank Accession Number O35682). In addition, the protein has 39 of 125 residues (31%) identical to, and 58 of 125 residues (46%) positive with the 153 residue human four transmembrane domain MAL T-lymphocyte maturation-associated protein (GenBank Accession Number P21145). The MAL protein is believed to act as a signaling receptor and transporter of water-soluble molecules and ions across the lipid bilayer. Alonso and Weissman 1987 Proc Natl Acad Sci U.S.A. 84(7): 1997-2000.

The breadth of expression of 4339264 transcript among a wide range of normal and cancerous tissues identified using quantitative real-time PCR (Example 11 and FIG. 23) suggests that the protein encoded by the 4339264 gene has a generalized role in cell homeostasis. Expression of 4339264 is elevated in select human cancer cell lines relative to the tissue of origin and elevated in some fetal tissue relative to the adult tissue, indicating a role in organogenesis and tissue repair. Overexpression of 4339264 should therefore contribute to tumor genesis. In addition, high expression of 4339264 in fetal kidney relative to the adult kidney suggests a likely role of 4339264 in organogenesis.

Successful therapeutic targeting and downregulation of 4339264 and/or its splice variants will result in significant anti-tumor activity, especially in combination with established cytotoxic/genotoxic therapies (*i.e.* chemosensitization, radiosensitization). Moreover successful therapeutic targeting of 4339264 will also limit the extent and frequency of metastatic dissemination (*i.e.*, tumor burden) and potentially limit tumor angiogenesis.

Therapeutic targeting of 4339264 and its splice variants will also be provided by generation of human or humanized monoclonal antibodies that block the ability of 4339264 to interact with cognate receptor(s) and elicit a transmembrane signal(s). Equally, the generation of small molecules (*i.e.*, synthetics, cell permeable peptides, other) that specifically interfere with one or more of the

downstream signaling components in the pathway(s) activated by 4339264 when bound to specific cognate receptor(s) will have significant anti-tumor activity as described above. Likewise, the introduction of antisense constructs (*i.e.*, naked DNA, adenoviral constructs) or ribozymes to inhibit the expression of 4339264 in select human malignancies will also have significant anti-tumor activity

5 as described above.

Clone 4339264 thus has diagnostic and therapeutic utility in pathologies related to cell signaling, regulation and development. Based on the expression profile of the 4339264 gene (Example 11 and FIG. 23) the therapeutic indications for targeting 4339264 include malignant melanomas, small cell lung carcinomas and renal cell carcinoma.

10 Nucleic Acids

One aspect of the invention pertains to isolated nucleic acid molecules (*i.e.*, SEQ ID NOs:1, 3, 5, 7, 9, 11, 13, 15, 17, 19, 21, 23, 25, 27, 74, 76, 78 and 80, plus 29 and 31) that encode the SECX polypeptides of the invention, wherein the SECX polypeptides are selected from the group comprising clone 2777610, clone 2864933-1, clone 2864933-2, clone 2982339, clone 3352358-1, clone 3352358-2, clone 3884846, clone 3884846-1, clone 3884846-2, clone 3911675, clone 3911675-2, clone 4004056, clone 4004056.0.143u, clone 4004731-1, clone 4009334-1, clone 4009334-2, clone 4035508, and clone 4339264 polypeptides, (*i.e.*, SEQ ID NOs:2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22, 24, 26, 28, 75, 77, 79 and 81, plus 30 and 32; see Table 1 and FIGS. 1-17), or biologically active portions thereof, as well as nucleic acid fragments sufficient for use as hybridization probes to identify 20 SECX-encoding nucleic acids (*e.g.*, SECX mRNA) and fragments for use as PCR primers for the amplification or mutation of SECX nucleic acid molecules. As used herein, the term "nucleic acid molecule" is intended to include DNA molecules (*e.g.*, cDNA or genomic DNA), RNA molecules (*e.g.*, mRNA), analogs of the DNA or RNA generated using nucleotide analogs, and derivatives, fragments and homologs thereof. The nucleic acid molecule can be single-stranded or 25 double-stranded, but preferably is double-stranded DNA. SECX nucleic acids of the invention include SEQ ID NOs:1, 3, 5, 7, 9, 11, 13, 15, 17, 19, 21, 23, 25, 27, 74, 76, 78 and 80, plus 29 and 31 (Table 1 and FIGS. 1-17), and fragments, homologs, and derivatives thereof.

30 "Probes" refer to nucleic acid sequences of variable length, preferably between at least about 10 nucleotides (nt), 100 nt, or as many as about, *e.g.*, 6,000 nt, depending on use. Probes are used in the detection of identical, similar, or complementary nucleic acid sequences. Longer length probes are usually obtained from a natural or recombinant source, are highly specific and much slower to hybridize than oligomers. Probes may be single- or double-stranded and designed to have specificity in PCR, membrane-based hybridization technologies, or ELISA-like technologies.

35 An "isolated" nucleic acid molecule is one that is separated from other nucleic acid molecules which are present in the natural source of the nucleic acid. Preferably, an "isolated" nucleic acid is free

of sequences which naturally flank the nucleic acid (*i.e.*, sequences located at the 5' and 3' ends of the nucleic acid) in the genomic DNA of the organism from which the nucleic acid is derived. For example, in various embodiments, an isolated nucleic acid molecule encoding any one of the SECX polypeptides, including chemokine receptor-like protein, semaphorin protein-like splice variants, a putative mitochondrial protein (clone 2982339), SLIT protein-like splice variants, a putative microbody (peroxisome) associated protein (clone 3884846), a tetraspanin-like protein, a putative proline-rich membrane protein (clone 4004056), a laminin β-chain precursor-like protein, AVENA protein-like splice variants (clones 4009334-1 and 4009334-2), a fetal lung-associated protein (clone 4035508) and a myeloid upregulated protein (clone 4339264), can contain less than about 5 kb, 4 kb, 3 kb, 2 kb, 1 kb, 0.5 kb or 0.1 kb of nucleotide sequences which naturally flank the nucleic acid molecule in genomic DNA of the cell from which the nucleic acid is derived (*e.g.*, adult and fetal cells from tissues including bone tissue (including bone marrow), heart, lymph node, pancreas, spleen, thymus, placenta, kidney, liver, thalamus, brain, pituitary, breast, lung, salivary gland and adrenal gland). Moreover, an "isolated" nucleic acid molecule, *e.g.*, a cDNA molecule, can be substantially free of other cellular material or culture medium when produced by recombinant techniques, or of chemical precursors or other chemicals when chemically synthesized.

A nucleic acid molecule of the present invention, *e.g.*, a SECX nucleic acid molecule having the nucleotide sequence of SEQ ID NOs:1, 3, 5, 7, 9, 11, 13, 15, 17, 19, 21, 23, 25 or 27, plus 29 or 31, or a complement of any of these nucleotide sequences, can be isolated using standard molecular biology techniques and the sequence information provided herein. Using all or a portion of the SECX nucleic acid sequences of SEQ ID NOs:1, 3, 5, 7, 9, 11, 13, 15, 17, 19, 21, 23, 25 or 27, plus 29 or 31, or a complement of any of these nucleotide sequences, as a hybridization probe, said SECX molecules can be isolated using standard hybridization and cloning techniques (*e.g.*, as described in Sambrook *et al.*, (eds.), MOLECULAR CLONING: A LABORATORY MANUAL 2nd Ed., Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY, 1989; and Ausubel, *et al.*, (eds.), CURRENT PROTOCOLS IN MOLECULAR BIOLOGY, John Wiley & Sons, New York, NY, 1993.)

A nucleic acid of the invention can be amplified using cDNA, mRNA or alternatively, genomic DNA, as a template and appropriate oligonucleotide primers according to standard PCR amplification techniques. The nucleic acid so amplified can be cloned into an appropriate vector and characterized by DNA sequence analysis. Furthermore, oligonucleotides corresponding to SECX nucleotide sequences can be prepared by standard synthetic techniques, *e.g.*, using an automated DNA synthesizer.

As used herein, the term "oligonucleotide" refers to a series of linked nucleotide residues, which oligonucleotide has a sufficient number of nucleotide bases to be used in a PCR reaction. A short oligonucleotide sequence may be based on, or designed from, a genomic or cDNA sequence and is used to amplify, confirm, or reveal the presence of an identical, similar or complementary DNA or

RNA in a particular cell or tissue. Oligonucleotides comprise portions of a nucleic acid sequence having about 10 nt, 50 nt, or 100 nucleotides in length, preferably about 15 nucleotides to 30 nucleotides in length. In one embodiment, an oligonucleotide comprising a nucleic acid molecule less than 100 nucleotides in length would further comprise at least 6 contiguous nucleotides of SEQ ID NOs:1, 3, 5, 7, 9, 11, 13, 15, 17, 19, 21, 23, 25, 27, 74, 76, 78 and 80, plus 29 and 31, or a complement thereof. Oligonucleotides may be chemically synthesized and may be used as probes.

In an embodiment, an isolated nucleic acid molecule of the invention comprises a SECX nucleic acid molecule that is a complement of the nucleotide sequence shown in SEQ ID NOs:1, 3, 5, 7, 9, 11, 13, 15, 17, 19, 21, 23, 25, 27, 74, 76, 78 and 80, plus 29 and 31, or a portion of this nucleotide sequence. A nucleic acid molecule that is complementary to said SECX nucleotide sequences is one that is sufficiently complementary to the nucleotide sequence shown in SEQ ID NOs:1, 3, 5, 7, 9, 11, 13, 15, 17, 19, 21, 23, 25, 27, 74, 76, 78 and 80, plus 29 and 31, or a portion of this nucleotide sequence, that it can hydrogen bond with little or no mismatches to the given SECX nucleotide sequence, thereby forming a stable duplex.

As used herein, the term "complementary" refers to Watson-Crick or Hoogsteen base pairing between nucleotides units of a nucleic acid molecule, and the term "binding" means the physical or chemical interaction between two polypeptides or compounds or associated polypeptides or compounds or combinations thereof. Binding includes ionic, non-ionic, Von der Waals, hydrophobic interactions, etc. A physical interaction can be either direct or indirect. Indirect interactions may be through or due to the effects of another polypeptide or compound. Direct binding refers to interactions that do not take place through, or due to, the effect of another polypeptide or compound, but instead are without other substantial chemical intermediates.

Moreover, the nucleic acid molecule of the invention can comprise only a portion of the nucleic acid sequence of SEQ ID NOs:1, 3, 5, 7, 9, 11, 13, 15, 17, 19, 21, 23, 25, 27, 74, 76, 78 and 80, plus 29 and 31, or the nucleotide sequence of the DNA insert of the plasmid, e.g., e.g., the pSecTag2 B and pSecV5His vectors described in Example 3, wherein e.g., a fragment that can be used as a probe or primer or a fragment encoding a biologically active portion of SECX. Fragments provided herein are defined as sequences of at least 6 (contiguous) nucleic acids or at least 4 (contiguous) amino acids, a length sufficient to allow for specific hybridization in the case of nucleic acids or for specific recognition of an epitope in the case of amino acids, respectively, and are at most some portion less than a full length sequence. Fragments may be derived from any contiguous portion of a nucleic acid or amino acid sequence of choice. Derivatives are nucleic acid sequences or amino acid sequences formed from the native compounds either directly or by modification or partial substitution. Analogs are nucleic acid sequences or amino acid sequences that have a structure similar to, but not identical to, the native compound but differs from it in respect to certain components or side chains. Analogs may be synthetic or from a different evolutionary origin and may have a similar or opposite metabolic

activity compared to wild type. Homologs are nucleic acid sequences or amino acid sequences of a particular gene that are derived from different species.

Derivatives and analogs may be full length or other than full length, if the derivative or analog contains a modified nucleic acid or amino acid, as described below. Derivatives or analogs of the 5 nucleic acids or proteins of the invention include, but are not limited to, molecules comprising regions that are substantially homologous to the nucleic acids or proteins of the invention, in various embodiments, by at least about 30%, 50%, 70%, 80%, or 95% identity (with a preferred identity of 80-95%) over a nucleic acid or amino acid sequence of identical size or when compared to an aligned sequence in which the alignment is done by a computer homology program known in the art (e.g., see 10 below), or whose encoding nucleic acid is capable of hybridizing to the complement of a sequence encoding the aforementioned proteins under stringent, moderately stringent, or low stringent conditions. See e.g. Ausubel, *et al.*, CURRENT PROTOCOLS IN MOLECULAR BIOLOGY, John Wiley & Sons, New York, NY, 1993, and below.

A "homologous nucleic acid sequence" or "homologous amino acid sequence," or variations 15 thereof, refer to sequences characterized by a homology at the nucleotide level or amino acid level as discussed above. Homologous nucleotide sequences encode those sequences coding for isoforms of SECX polypeptide. Isoforms can be expressed in different tissues of the same organism as a result of, for example, alternative splicing of RNA. Alternatively, isoforms can be encoded by different genes. In the present invention, homologous nucleotide sequences include nucleotide sequences encoding for 20 a SECX polypeptide of species other than humans, including, but not limited to, mammals, and thus can include, e.g., mouse, rat, rabbit, dog, cat cow, horse, and other organisms. Homologous nucleotide sequences also include, but are not limited to, naturally occurring allelic variations and mutations of the nucleotide sequences set forth herein. A homologous nucleotide sequence does not, however, include the nucleotide sequence encoding human SECX protein. Homologous nucleic acid sequences 25 include those nucleic acid sequences that encode conservative amino acid substitutions (see below) in SEQ ID NOS:1, 3, 5, 7, 9, 11, 13, 15, 17, 19, 21, 23, 25, 27, 74, 76, 78 and 80, plus 29 and 31, as well as a polypeptide having SECX activity. Biological activities of the individual SECX proteins are described above. A homologous amino acid sequence does not encode the amino acid sequence of a human SECX polypeptide.

30 A SECX polypeptide is encoded by the open reading frame ("ORF") of a SECX nucleic acid. The invention includes the nucleic acid sequence comprising the stretch of nucleic acid sequences of SEQ ID NOS:1, 3, 5, 7, 9, 11, 13, 15, 17, 19, 21, 23, 25, 27, 74, 76, 78 and 80, plus 29 and 31, that comprises the ORF of that nucleic acid sequence and encodes a polypeptide of SEQ ID NOS:2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22, 24, 26, 28, 75, 77, 79 and 81, plus 30 and 32.

35 An "open reading frame" ("ORF") corresponds to a nucleotide sequence that could potentially be translated into a polypeptide. A stretch of nucleic acids comprising an ORF is uninterrupted by a

stop codon. An ORF that represents the coding sequence for a full protein begins with an ATG "start" codon and terminates with one of the three "stop" codons, namely, TAA, TAG, or TGA. For the purposes of this invention, an ORF may be any part of a coding sequence, with or without a start codon, a stop codon, or both. For an ORF to be considered as a good candidate for coding for a bona fide cellular protein, a minimum size requirement is often set, for example, a stretch of DNA that would encode a protein of 50 amino acids or more.

The nucleotide sequence determined from the cloning of the human SECX gene allows for the generation of probes and primers designed for use in identifying and/or cloning SECX homologues in other cell types, e.g. from other tissues, as well as SECX homologues from other mammals. The probe/primer typically comprises substantially purified oligonucleotide. The oligonucleotide typically comprises a region of nucleotide sequence that hybridizes under stringent conditions to at least about 12, 25, 50, 100, 150, 200, 250, 300, 350 or 400 consecutive sense strand nucleotide sequence of SEQ ID NOs:1, 3, 5, 7, 9, 11, 13, 15, 17, 19, 21, 23, 25, 27, 74, 76, 78 and 80, plus 29 and 31, or the nucleotide sequence of the DNA insert of the plasmid such as, e.g., the pSecTag2 B and pSecV5His vectors described in Example 3; or an anti-sense strand nucleotide sequence of a SECX nucleotide or the anti-sense strand SECX nucleotide sequence of the DNA insert of the plasmid known in the art; or of a naturally occurring mutant of a SECX nucleotide, or the naturally occurring mutant of the DNA insert of the plasmid vector known in the art.

Probes based on the human SECX nucleotide sequence can be used to detect transcripts or genomic sequences encoding the same or homologous proteins. In various embodiments, the probe further comprises a label group attached thereto, e.g. the label group can be a radioisotope, a fluorescent compound, an enzyme, or an enzyme co-factor. Such probes can be used as a part of a diagnostic test kit for identifying cells or tissue which misexpress a SECX protein, e.g., by measuring a level of a SECX-encoding nucleic acid in a sample of cells from a subject e.g., detecting SECX mRNA levels or determining whether a genomic SECX gene has been mutated or deleted.

"A polypeptide having a biologically active portion of SECX" refers to polypeptides exhibiting activity similar, but not necessarily identical to, an activity of a polypeptide of the present invention, including mature forms, as measured in a particular biological assay, with or without dose dependency. A nucleic acid fragment encoding a "biologically active portion of SECX" can be prepared by isolating a portion of a SECX nucleotide that encodes a polypeptide having a SECX biological activity (wherein the biological activities of the SECX proteins are described above), expressing the encoded portion of SECX protein (e.g., by recombinant expression *in vitro*) and assessing the activity of the encoded portion of SECX. For example, a nucleic acid fragment encoding a biologically active portion of SECX includes an extracellular domain, e.g., the clone 2864933-1 amino acid residues 19 to 644 of SEQ ID NO:4. In another embodiment, a nucleic acid fragment encoding a biologically active portion of SECX that includes an extracellular domain includes the

DNA encoding such domains, e.g., at least the nucleic acids of SEQ ID NO:9 that encodes the human clone 3352358-1 extracellular domain represented by amino acid residues 42 to 486 of SEQ ID NO:10.

SECX variants

5 The invention further encompasses any one or more nucleic acid molecules that differ from the SECX nucleotide sequence shown in at least one of SEQ ID NOs:1, 3, 5, 7, 9, 11, 13, 15, 17, 19, 21, 23, 25, 27, 74, 76, 78 and 80, plus 29 and 31, due to degeneracy of the genetic code and thus encode the same SECX protein as that encoded by any of the above nucleotide sequences. In another embodiment, an isolated SECX nucleic acid molecule of the invention has a nucleotide sequence 10 encoding a protein having any one amino acid sequence shown in SEQ ID NOs:2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22, 24, 26, 28, 75, 77, 79 and 81, plus 30 and 32.

In addition to these human SECX nucleotide sequences, or the SECX nucleotide sequence of the DNA insert of a plasmid or vector, it will be appreciated by those skilled in the art that DNA sequence polymorphisms that lead to changes in the amino acid sequences of a SECX may exist 15 within a population (e.g., the human population). Such genetic polymorphism in a SECX gene may exist among individuals within a population due to natural allelic variation. As used herein, the terms "gene" and "recombinant gene" refer to nucleic acid molecules comprising an open reading frame encoding a SECX protein, preferably a mammalian SECX protein. Such natural allelic variations can typically result in 1-5% variance in the nucleotide sequence of the SECX gene. Any and all such 20 nucleotide variations and resulting amino acid polymorphisms in SECX that are the result of natural allelic variation and that do not alter the functional activity of SECX are intended to be within the scope of the invention.

Moreover, nucleic acid molecules encoding SECX proteins from other species, and thus that have a nucleotide sequence that differs from the human sequence disclosed herein, are intended to be 25 within the scope of the invention. Nucleic acid molecules corresponding to natural allelic variants and homologues of a SECX cDNAs of the invention can be isolated based on their homology to the human SECX nucleic acids disclosed herein using the human cDNAs, or a portion thereof, as a hybridization probe according to standard hybridization techniques under stringent hybridization conditions. For example, a soluble human SECX cDNA can be isolated based on its homology to human 30 membrane-bound SECX. Likewise, a membrane-bound human SECX cDNA can be isolated based on its homology to soluble human SECX.

Accordingly, in another embodiment, an isolated nucleic acid molecule of the invention is at least 6 nucleotides in length and hybridizes under stringent conditions to the nucleic acid molecule comprising at least one SECX nucleotide sequence. In another embodiment, the nucleic acid is at least 35 10, 25, 50, 100, 250, 500 or 2000 nucleotides in length. In another embodiment, an isolated nucleic

acid molecule of the invention hybridizes to the coding region. As used herein, the term "hybridizes under stringent conditions" is intended to describe conditions for hybridization and washing under which nucleotide sequences at least 60% homologous to each other typically remain hybridized to each other.

5 Homologs (*i.e.*, nucleic acids encoding SECX proteins derived from species other than human) or other related sequences (*e.g.*, paralogs) can be obtained by low, moderate or high stringency hybridization with all or a portion of the particular human sequence as a probe using methods well known in the art for nucleic acid hybridization and cloning.

As used herein, the phrase "stringent hybridization conditions" refers to conditions under
10 which a probe, primer or oligonucleotide will hybridize to its target sequence, but to no other sequences. Stringent conditions are sequence-dependent and will be different in different circumstances. Longer sequences hybridize specifically at higher temperatures than shorter sequences. Generally, stringent conditions are selected to be about 5°C lower than the thermal melting point (Tm) for the specific sequence at a defined ionic strength and pH. The Tm is the temperature (under defined
15 ionic strength, pH and nucleic acid concentration) at which 50% of the probes complementary to the target sequence hybridize to the target sequence at equilibrium. Since the target sequences are generally present at excess, at Tm, 50% of the probes are occupied at equilibrium. Typically, stringent conditions will be those in which the salt concentration is less than about 1.0 M sodium ion, typically about 0.01 to 1.0 M sodium ion (or other salts) at pH 7.0 to 8.3 and the temperature is at least about
20 30°C for short probes, primers or oligonucleotides (*e.g.*, 10 nt to 50 nt) and at least about 60°C for longer probes, primers and oligonucleotides. Stringent conditions may also be achieved with the addition of destabilizing agents, such as formamide.

Stringent conditions are known to those skilled in the art and can be found in Ausubel *et al.*,
25 (eds.), CURRENT PROTOCOLS IN MOLECULAR BIOLOGY, John Wiley & Sons, N.Y. (1989), 6.3.1-6.3.6. Preferably, the conditions are such that sequences at least about 65%, 70%, 75%, 85%, 90%, 95%, 98%, or 99% homologous to each other typically remain hybridized to each other. A non-limiting example of stringent hybridization conditions are hybridization in a high salt buffer comprising 6X SSC, 50 mM Tris-HCl (pH 7.5), 1 mM EDTA, 0.02% PVP, 0.02% Ficoll, 0.02% BSA, and 500 mg/ml denatured salmon sperm DNA at 65°C, followed by one or more washes in 0.2X SSC, 0.01% BSA at
30 50°C. An isolated nucleic acid molecule of the invention that hybridizes under stringent conditions to a SECX nucleotide sequence corresponds to a naturally-occurring nucleic acid molecule. As used herein, a "naturally-occurring" nucleic acid molecule refers to an RNA or DNA molecule having a nucleotide sequence that occurs in nature (*e.g.*, encodes a natural protein).

In a second embodiment, a nucleic acid sequence that is hybridizable to at least one SECX
35 nucleic acid molecule, or fragments, analogs or derivatives thereof, under conditions of moderate stringency is provided. A non-limiting example of moderate stringency hybridization conditions are

hybridization in 6X SSC, 5X Denhardt's solution, 0.5% SDS and 100 mg/ml denatured salmon sperm DNA at 55°C, followed by one or more washes in 1X SSC, 0.1% SDS at 37°C. Other conditions of moderate stringency that may be used are well-known in the art. See, e.g., Ausubel *et al.* (eds.), 1993, CURRENT PROTOCOLS IN MOLECULAR BIOLOGY, John Wiley & Sons, NY, and Kriegler, 1990, GENE TRANSFER AND EXPRESSION, A LABORATORY MANUAL, Stockton Press, NY.

In a third embodiment, a nucleic acid that is hybridizable to at least one SECX nucleic acid molecule, or fragments, analogs or derivatives thereof, under conditions of low stringency, is provided. A non-limiting example of low stringency hybridization conditions are hybridization in 35% formamide, 5X SSC, 50 mM Tris-HCl (pH 7.5), 5 mM EDTA, 0.02% PVP, 0.02% Ficoll, 0.2% BSA, 100 mg/ml denatured salmon sperm DNA, 10% (wt/vol) dextran sulfate at 40°C, followed by one or more washes in 2X SSC, 25 mM Tris-HCl (pH 7.4), 5 mM EDTA, and 0.1% SDS at 50°C. Other conditions of low stringency that may be used are well known in the art (e.g., as employed for cross-species hybridizations). See, e.g., Ausubel *et al.* (eds.), 1993, CURRENT PROTOCOLS IN MOLECULAR BIOLOGY, John Wiley & Sons, NY, and Kriegler, 1990, GENE TRANSFER AND EXPRESSION, A LABORATORY MANUAL, Stockton Press, NY; Shilo and Weinberg, 1981, *Proc Natl Acad Sci USA* 78: 6789-6792.

Conservative mutations

In addition to naturally-occurring allelic variants of the SECX sequence that may exist in the population, the skilled artisan will further appreciate that changes can be introduced by mutation into at least one SECX nucleotide sequence of SEQ ID NOs:1, 3, 5, 7, 9, 11, 13, 15, 17, 19, 21, 23, 25, 27, 20 27, 74, 76, 78 and 80, plus 29 and 31, thereby leading to changes in the amino acid sequence of the encoded SECX protein, without altering the functional ability of the SECX protein. For example, nucleotide substitutions leading to amino acid substitutions at "non-essential" amino acid residues can be made in the sequence of SEQ ID NOs:2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22, 24, 26, 28, 75, 77, 79 25 and 81, plus 30 and 32, or the SECX nucleotide sequence of the DNA insert of the plasmid or vector known in the art. A "non-essential" amino acid residue is a residue that can be altered from the wild-type sequence of SECX without altering the biological activity, whereas an "essential" amino acid residue is required for biological activity. For example, amino acid residues that are conserved among the SECX proteins of the present invention, are predicted to be particularly unamenable to alteration.

Another aspect of the invention pertains to nucleic acid molecules encoding SECX proteins that contain changes in amino acid residues that are not essential for activity. Such SECX proteins differ in amino acid sequence from SEQ ID NOs:2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22, 24, 26, 28, 75, 77, 79 and 81, plus 30 and 32, yet retain biological activity. In one embodiment, the isolated nucleic acid molecule comprises a nucleotide sequence encoding a protein, wherein the protein comprises an

amino acid sequence at least about 45% homologous to at least one SECX amino acid sequence. Preferably, the protein encoded by the nucleic acid molecule is at least about 60% homologous to at least one SECX polypeptide, more preferably at least about 70% homologous, at least about 80% homologous, at least about 90% homologous, and most preferably at least about 95% homologous to that given SECX polypeptide.

5 An isolated nucleic acid molecule encoding a SECX protein homologous to a given SECX protein can be created by introducing one or more nucleotide substitutions, additions or deletions into the corresponding SECX nucleotide sequence, such that one or more amino acid substitutions, additions or deletions are introduced into the encoded protein.

10 Mutations can be introduced into SEQ ID NOs:1, 3, 5, 7, 9, 11, 13, 15, 17, 19, 21, 23, 25, 27, 74, 76, 78 and 80, plus 29 and 31, by standard techniques, e.g., site-directed mutagenesis and PCR-mediated mutagenesis. Preferably, conservative amino acid substitutions are made at one or more predicted non-essential amino acid residues. A "conservative amino acid substitution" is one in which the amino acid residue is replaced with an amino acid residue having a similar side chain.

15 Families of amino acid residues having similar side chains have been defined in the art. These families include amino acids with basic side chains (e.g., lysine, arginine, histidine), acidic side chains (e.g., aspartic acid, glutamic acid), uncharged polar side chains (e.g., glycine, asparagine, glutamine, serine, threonine, tyrosine, cysteine), nonpolar side chains (e.g., alanine, valine, leucine, isoleucine, proline, phenylalanine, methionine, tryptophan), beta-branched side chains (e.g., threonine, valine, isoleucine) and aromatic side chains (e.g., tyrosine, phenylalanine, tryptophan, histidine). Thus, a predicted nonessential amino acid residue in SECX is replaced with another amino acid residue from the same side chain family. Alternatively, in another embodiment, mutations can be introduced randomly along all or part of a SECX coding sequence, e.g., by saturation mutagenesis, and the resultant mutants can be screened for SECX biological activity to identify mutants that retain activity. Following 20 mutagenesis, the encoded SECX protein can be expressed by any recombinant technology known in the art and the activity of the protein can be determined.

25 In one embodiment, a mutant SECX protein can be assayed for (1) the ability to form protein:protein interactions with other SECX proteins, other cell-surface proteins, or biologically active portions thereof, (2) complex formation between a mutant SECX protein and a SECX ligand; (3) the ability of a mutant SECX protein to bind to an intracellular target protein or biologically active portion thereof; (e.g. avidin proteins).

Antisense

30 Another aspect of the invention pertains to isolated antisense nucleic acid molecules that are hybridizable to or complementary to a SECX nucleic acid molecule, or fragments, analogs or derivatives thereof. An "antisense" nucleic acid comprises a nucleotide sequence that is

complementary to a "sense" nucleic acid encoding a protein, e.g., complementary to the coding strand of a double-stranded cDNA molecule or complementary to an mRNA sequence. In specific aspects, antisense nucleic acid molecules are provided that comprise a sequence complementary to at least about 10, 25, 50, 100, 250 or 500 nucleotides or an entire SECX coding strand, or to only a portion thereof. Nucleic acid molecules encoding fragments, homologs, derivatives and analogs of a SECX protein, or antisense nucleic acids complementary to a SECX nucleic acid sequence, are additionally provided.

In one embodiment, an antisense nucleic acid molecule is antisense to a "coding region" of the coding strand of a nucleotide sequence encoding SECX. The term "coding region" refers to the region of the nucleotide sequence comprising codons which are translated into amino acid residues (e.g., ORFs shown in FIGS. 1-17). In another embodiment, the antisense nucleic acid molecule is antisense to a "noncoding region" of the coding strand of a nucleotide sequence encoding SECX. The term "noncoding region" refers to 5' and 3' sequences which flank the coding region that are not translated into amino acids (i.e., also referred to as 5' and 3' untranslated regions).

Given the coding strand sequences encoding SECX disclosed herein (e.g., SEQ ID NOs:1, 3, 5, 7, 9, 11, 13, 15, 17, 19, 21, 23, 25, 27, 74, 76, 78 and 80, plus 29 and 31), antisense nucleic acids of the invention can be designed according to the rules of Watson and Crick or Hoogsteen base pairing. The antisense nucleic acid molecule can be complementary to the entire coding region of SECX mRNA, but more preferably is an oligonucleotide that is antisense to only a portion of the coding or noncoding region of SECX mRNA. For example, the antisense oligonucleotide can be complementary to the region surrounding the translation start site of SECX mRNA. An antisense oligonucleotide can be, for example, about 5, 10, 15, 20, 25, 30, 35, 40, 45 or 50 nucleotides in length. An antisense nucleic acid of the invention can be constructed using chemical synthesis or enzymatic ligation reactions using procedures known in the art. For example, an antisense nucleic acid (e.g., an antisense oligonucleotide) can be chemically synthesized using naturally occurring nucleotides or variously modified nucleotides designed to increase the biological stability of the molecules or to increase the physical stability of the duplex formed between the antisense and sense nucleic acids, e.g., phosphorothioate derivatives and acridine substituted nucleotides can be used.

Examples of modified nucleotides that can be used to generate the antisense nucleic acid include: 5-fluorouracil, 5-bromouracil, 5-chlorouracil, 5-iodouracil, hypoxanthine, xanthine, 4-acetylcytosine, 5-(carboxyhydroxymethyl) uracil, 5-carboxymethylaminomethyl-2-thiouridine, 5-carboxymethylaminomethyluracil, dihydrouracil, beta-D-galactosylqueosine, inosine, N6-isopentenyladenine, 1-methylguanine, 1-methylinosine, 2,2-dimethylguanine, 2-methyladenine, 2-methylguanine, 3-methylcytosine, 5-methylcytosine, N6-adenine, 7-methylguanine, 5-methylaminomethyluracil, 5-methoxyaminomethyl-2-thiouracil, beta-D-mannosylqueosine, 5'-methoxycarboxymethyluracil, 5-methoxyuracil, 2-methylthio-N6-isopentenyladenine,

uracil-5-oxyacetic acid (v), wybutoxosine, pseudouracil, queosine, 2-thiocytosine, 5-methyl-2-thiouracil, 2-thiouracil, 4-thiouracil, 5-methyluracil, uracil-5-oxyacetic acid methylester, uracil-5-oxyacetic acid (v), 5-methyl-2-thiouracil, 3-(3-amino-3-N-2-carboxypropyl) uracil, (acp3)w, and 2,6-diaminopurine. Alternatively, the antisense nucleic acid can be produced biologically using an expression vector into which a nucleic acid has been subcloned in an antisense orientation (*i.e.*, RNA transcribed from the inserted nucleic acid will be of an antisense orientation to a target nucleic acid of interest, described further in the following subsection).

The antisense nucleic acid molecules of the invention are typically administered to a subject or generated *in situ* such that they hybridize with or bind to cellular mRNA and/or genomic DNA encoding a SECX protein to thereby inhibit expression of the protein, *e.g.*, by inhibiting transcription and/or translation. The hybridization can be by conventional nucleotide complementarity to form a stable duplex, or, for example, in the case of an antisense nucleic acid molecule that binds to DNA duplexes, through specific interactions in the major groove of the double helix. An example of a route of administration of antisense nucleic acid molecules of the invention includes direct injection at a tissue site. Alternatively, antisense nucleic acid molecules can be modified to target selected cells and then administered systemically. For example, for systemic administration, antisense molecules can be modified such that they specifically bind to receptors or antigens expressed on a selected cell surface, *e.g.*, by linking the antisense nucleic acid molecules to peptides or antibodies that bind to cell surface receptors or antigens. The antisense nucleic acid molecules can also be delivered to cells using the vectors described herein. To achieve sufficient intracellular concentrations of antisense molecules, vector constructs in which the antisense nucleic acid molecule is placed under the control of a strong pol II or pol III promoter are preferred.

In yet another embodiment, the antisense nucleic acid molecule of the invention is an α -anomeric nucleic acid molecule. An α -anomeric nucleic acid molecule forms specific double-stranded hybrids with complementary RNA in which, contrary to the usual β -units, the strands run parallel to each other (Gaultier *et al.* (1987) *Nucleic Acids Res* 15: 6625-6641). The antisense nucleic acid molecule can also comprise a 2'-o-methylribonucleotide (Inoue *et al.* (1987) *Nucleic Acids Res* 15: 6131-6148) or a chimeric RNA-DNA analogue (Inoue *et al.* (1987) *FEBS Lett* 215: 327-330).

Ribozymes and PNA moieties

Nucleic acid modifications include, by way of nonlimiting example, modified bases, and nucleic acids whose sugar phosphate backbones are modified or derivatized. These modifications are carried out at least in part to enhance the chemical stability of the modified nucleic acid, such that they may be used, for example, as antisense binding nucleic acids in therapeutic applications in a subject.

In one embodiment, an antisense nucleic acid of the invention is a ribozyme. Ribozymes are catalytic RNA molecules with ribonuclease activity that are capable of cleaving a single-stranded

nucleic acid, *e.g.*, an mRNA, to which they have a complementary region. Thus, ribozymes (*e.g.*, hammerhead ribozymes (described in Haselhoff and Gerlach (1988) *Nature* 334:585-591)) can be used to catalytically cleave SECX mRNA transcripts to thereby inhibit translation of SECX mRNA. A ribozyme having specificity for a SECX-encoding nucleic acid can be designed based upon the 5 nucleotide sequence of a SECX cDNA disclosed herein (*i.e.*, SEQ ID NOS:1, 3, 5, 7, 9, 11, 13, 15, 17, 19, 21, 23, 25, 27, 74, 76, 78 and 80, plus 29 and 31). For example, a derivative of a Tetrahymena L-19 IVS RNA can be constructed in which the nucleotide sequence of the active site is complementary to the nucleotide sequence to be cleaved in a SECX-encoding mRNA. See, *e.g.*, Cech 10 *et al.* U.S. Pat. No. 4,987,071; and Cech *et al.* U.S. Pat. No. 5,116,742. Alternatively, SECX mRNA can be used to select a catalytic RNA having a specific ribonuclease activity from a pool of RNA molecules. See, *e.g.*, Bartel *et al.*, (1993) *Science* 261:1411-1418.

Alternatively, SECX gene expression can be inhibited by targeting nucleotide sequences complementary to the regulatory region of a SECX gene (*e.g.*, the SECX promoter and/or enhancers) to form triple helical structures that prevent transcription of the SECX gene in target cells. See 15 generally, Helene. (1991) *Anticancer Drug Des.* 6: 569-84; Helene. *et al.* (1992) *Ann. N.Y. Acad. Sci.* 660:27-36; and Maher (1992) *Bioassays* 14: 807-15.

In various embodiments, the nucleic acids of SECX can be modified at the base moiety, sugar moiety or phosphate backbone to improve, *e.g.*, the stability, hybridization, or solubility of the molecule. For example, the deoxyribose phosphate backbone of the nucleic acids can be modified to 20 generate peptide nucleic acids (see Hyrup *et al.* (1996) *Bioorg Med Chem* 4: 5-23). As used herein, the terms "peptide nucleic acids" or "PNAs" refer to nucleic acid mimics, *e.g.*, DNA mimics, in which the deoxyribose phosphate backbone is replaced by a pseudopeptide backbone and only the four natural 25 nucleobases are retained. The neutral backbone of PNAs has been shown to allow for specific hybridization to DNA and RNA under conditions of low ionic strength. The synthesis of PNA oligomers can be performed using standard solid phase peptide synthesis protocols as described in Hyrup *et al.* (1996) above; Perry-O'Keefe *et al.* (1996) *PNAS* 93: 14670-675.

PNAs of SECX can be used in therapeutic and diagnostic applications. For example, PNAs can be used as antisense or antigene agents for sequence-specific modulation of gene expression by, *e.g.*, inducing transcription or translation arrest or inhibiting replication. PNAs of SECX can also be 30 used, *e.g.*, in the analysis of single base pair mutations in a gene by, *e.g.*, PNA directed PCR clamping; as artificial restriction enzymes when used in combination with other enzymes, *e.g.*, S1 nucleases (Hyrup B. (1996) above); or as probes or primers for DNA sequence and hybridization (Hyrup *et al.* (1996), above; Perry-O'Keefe (1996), above).

In another embodiment, PNAs of SECX can be modified, *e.g.*, to enhance their stability or 35 cellular uptake, by attaching lipophilic or other helper groups to PNA, by the formation of PNA-DNA chimeras, or by the use of liposomes or other techniques of drug delivery known in the art. For

example, PNA-DNA chimeras of SECX can be generated that may combine the advantageous properties of PNA and DNA. Such chimeras allow DNA recognition enzymes, e.g., RNase H and DNA polymerases, to interact with the DNA portion while the PNA portion would provide high binding affinity and specificity. PNA-DNA chimeras can be linked using linkers of appropriate lengths selected in terms of base stacking, number of bonds between the nucleobases, and orientation (Hyrup (1996) above). The synthesis of PNA-DNA chimeras can be performed as described in Hyrup (1996) above and Finn *et al.* (1996) *Nucl Acids Res* 24: 3357-63. For example, a DNA chain can be synthesized on a solid support using standard phosphoramidite coupling chemistry, and modified nucleoside analogs, e.g., 5'-(4-methoxytrityl)amino-5'-deoxy-thymidine phosphoramidite, can be used between the PNA and the 5' end of DNA (Mag *et al.* (1989) *Nucl Acid Res* 17: 5973-88). PNA monomers are then coupled in a stepwise manner to produce a chimeric molecule with a 5' PNA segment and a 3' DNA segment (Finn *et al.* (1996) above). Alternatively, chimeric molecules can be synthesized with a 5' DNA segment and a 3' PNA segment. See, Petersen *et al.* (1975) *Bioorg Med Chem Lett* 5: 1119-1124.

In other embodiments, the oligonucleotide may include other appended groups such as peptides (e.g., for targeting host cell receptors *in vivo*), or agents facilitating transport across the cell membrane (see, e.g., Letsinger *et al.*, 1989, *Proc. Natl. Acad. Sci. U.S.A.* 86:6553-6556; Lemaitre *et al.*, 1987, *Proc. Natl. Acad. Sci.* 84:648-652; PCT Publication No. W088/09810) or the blood-brain barrier (see, e.g., PCT Publication No. W089/10134). In addition, oligonucleotides can be modified with hybridization triggered cleavage agents (See, e.g., Krol *et al.*, 1988, *BioTechniques* 6:958-976) or intercalating agents. (See, e.g., Zon, 1988, *Pharm. Res.* 5: 539-549). To this end, the oligonucleotide may be conjugated to another molecule, e.g., a peptide, a hybridization triggered cross-linking agent, a transport agent, a hybridization-triggered cleavage agent, etc.

SECX proteins

The novel protein of the invention includes the SECX proteins whose sequences are provided in FIGS. 1-15 and 17 (SEQ ID NOs:2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22, 24, 26, 28, 75, 77, 79 and 81, plus 30 and 32). The invention also includes a mutant or variant protein, any of whose residues may be changed from the corresponding residue shown in FIG. 1-15 and 17 while still encoding a protein that maintains its SECX activities and physiological functions, or a functional fragment thereof. In the mutant or variant protein, up to 20% or more of the residues may be so changed.

In general, an SECX variant that preserves SECX-like function includes any variant in which residues at a particular position in the sequence have been substituted by other amino acids, and further include the possibility of inserting an additional residue or residues between two residues of the parent protein as well as the possibility of deleting one or more residues from the parent sequence.

Any amino acid substitution, insertion, or deletion is encompassed by the invention. In favorable circumstances, the substitution is a conservative substitution as defined above.

One aspect of the invention pertains to isolated SECX proteins, and biologically active portions thereof, or derivatives, fragments, analogs or homologs thereof. Also provided are
5 polypeptide fragments suitable for use as immunogens to raise anti-SECX antibodies. In one embodiment, native SECX proteins can be isolated from cells or tissue sources by an appropriate purification scheme using standard protein purification techniques. In another embodiment, SECX proteins are produced by recombinant DNA techniques. Alternative to recombinant expression, a SECX protein or polypeptide can be synthesized chemically using standard peptide synthesis
10 techniques.

An "isolated" or "purified" protein or biologically active portion thereof is substantially free of cellular material or other contaminating proteins from the cell or tissue source from which the SECX protein is derived, or substantially free from chemical precursors or other chemicals when chemically synthesized. The language "substantially free of cellular material" includes preparations of SECX
15 protein in which the protein is separated from cellular components of the cells from which it is isolated or recombinantly produced. In one embodiment, the language "substantially free of cellular material" includes preparations of SECX protein having less than about 30% (by dry weight) of non-SECX protein (also referred to herein as a "contaminating protein"), more preferably less than about 20% of non-SECX protein, still more preferably less than about 10% of non-SECX protein, and
20 most preferably less than about 5% non-SECX protein. When the SECX protein or biologically active portion thereof is recombinantly produced, it is also preferably substantially free of culture medium, *i.e.*, culture medium represents less than about 20%, more preferably less than about 10%, and most preferably less than about 5% of the volume of the protein preparation.

The language "substantially free of chemical precursors or other chemicals" includes
25 preparations of SECX protein in which the protein is separated from chemical precursors or other chemicals that are involved in the synthesis of the protein. In one embodiment, the language "substantially free of chemical precursors or other chemicals" includes preparations of SECX protein having less than about 30% (by dry weight) of chemical precursors or non-SECX chemicals, more preferably less than about 20% chemical precursors or non-SECX chemicals, still more preferably less
30 than about 10% chemical precursors or non-SECX chemicals, and most preferably less than about 5% chemical precursors or non-SECX chemicals.

Biologically active portions of a SECX protein include peptides comprising amino acid sequences sufficiently homologous to or derived from the amino acid sequence of the SECX protein, e.g., the amino acid sequence shown in SEQ ID NOs:2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22, 24, 26, 28,
35 75, 77, 79 and 81, plus 30 and 32, that include fewer amino acids than the full length SECX proteins, and exhibit at least one activity of a SECX protein. Typically, biologically active portions comprise a

domain or motif with at least one activity of the SECX protein. A biologically active portion of a SECX protein can be a polypeptide which is, for example, 10, 25, 50, 100 or more amino acids in length.

It is to be understood that a biologically active portion of a SECX protein of the present invention may contain at least one of the structural domains identified in Sections 1-14, above. An alternative biologically active portion of a SECX protein may contain an extracellular domain of the SECX protein. Another biologically active portion of a SECX protein may contain the transmembrane domain of the SECX protein. Yet another biologically active portion of a SECX protein of the present invention may contain the intracellular domain of the SECX protein.

Moreover, other biologically active portions, in which other regions of the protein are deleted, can be prepared by recombinant techniques and evaluated for one or more of the functional activities of a native SECX protein.

In an embodiment, the SECX protein has any one or more amino acid sequences shown in SEQ ID NOs:2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22, 24, 26, 28, 75, 77, 79 and 81, plus 30 and 32. In other embodiments, the SECX protein is substantially homologous to any one of SEQ ID NOs:2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22, 24, 26, 28, 75, 77, 79 and 81, plus 30 and 32, and retains the functional activity of that given SECX protein yet differs in amino acid sequence due to natural allelic variation or mutagenesis, as described in detail below. Accordingly, in another embodiment, the SECX protein is a protein that comprises an amino acid sequence at least about 75% homologous to any one amino acid sequence of SEQ ID NOs:2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22, 24, 26, 28, 75, 77, 79 and 81, plus 30 and 32, and retains the functional activity of that SECX protein.

This invention further features isolated SECX protein, or derivatives, fragments, analogs or homologs thereof, that is encoded by a nucleic acid molecule having a nucleotide sequence that hybridizes under stringent hybridization conditions to a nucleic acid molecule comprising the nucleotide sequence of any one or more of SEQ ID NOs:1, 3, 5, 7, 9, 11, 13, 15, 17, 19, 21, 23, 25, 27, 74, 76, 78 and 80, plus 29 and 31.

Determining homology between two or more sequences

To determine the percent homology of two amino acid sequences or of two nucleic acids, the sequences are aligned for optimal comparison purposes (*e.g.*, gaps can be introduced in the sequence of a first amino acid or nucleic acid sequence for optimal alignment with a second amino or nucleic acid sequence). The amino acid residues or nucleotides at corresponding amino acid positions or nucleotide positions are then compared. When a position in the first sequence is occupied by the same amino acid residue or nucleotide as the corresponding position in the second sequence, then the molecules are homologous at that position (*i.e.*, as used herein amino acid or nucleic acid "homology" is equivalent to amino acid or nucleic acid "identity").

The nucleic acid sequence homology may be determined as the degree of identity between two sequences. The homology may be determined using computer programs known in the art, such as GAP software provided in the GCG program package. See, Needleman and Wunsch 1970 *J Mol Biol* 48: 443-453. Using GCG GAP software with the following settings for nucleic acid sequence comparison:

- 5 GAP creation penalty of 5.0 and GAP extension penalty of 0.3, the coding region of the analogous nucleic acid sequences referred to above exhibits a degree of identity preferably of at least 70%, 75%, 80%, 85%, 90%, 95%, 98%, or 99%, with the CDS (*i.e.*, encoding) part of the DNA sequence shown in any one or more of SEQ ID NOS:1, 3, 5, 7, 9, 11, 13, 15, 17, 19, 21, 23, 25, 27, 74, 76, 78 and 80, plus 29 and 31.
- 10 The term "sequence identity" refers to the degree to which two polynucleotide or polypeptide sequences are identical on a residue-by-residue basis over a particular region of comparison. The term "percentage of sequence identity" is calculated by comparing two optimally aligned sequences over that region of comparison, determining the number of positions at which the identical nucleic acid base (*e.g.*, A, T, C, G, U, or I, in the case of nucleic acids) occurs in both sequences to yield the 15 number of matched positions, dividing the number of matched positions by the total number of positions in the region of comparison (*i.e.*, the window size), and multiplying the result by 100 to yield the percentage of sequence identity. The term "substantial identity" as used herein denotes a characteristic of a polynucleotide sequence, wherein the polynucleotide comprises a sequence that has at least 80 percent sequence identity, preferably at least 85 percent identity and often 90 to 95 percent 20 sequence identity, more usually at least 99 percent sequence identity as compared to a reference sequence over a comparison region. Similar calculation are used when comparing amino acid residues in polypeptide sequences.

Chimeric and fusion proteins

- The invention also provides SECX chimeric or fusion proteins. As used herein, a SECX 25 "chimeric protein" or "fusion protein" comprises a SECX polypeptide operatively linked to a non-SECX polypeptide. A "SECX polypeptide" refers to a polypeptide having an amino acid sequence corresponding to SECX, whereas a "non-SECX polypeptide" refers to a polypeptide having an amino acid sequence corresponding to a protein that is not substantially homologous to the SECX protein, *e.g.*, a protein that is different from the SECX protein and that is derived from the same or a different 30 organism. Within a SECX fusion protein the SECX polypeptide can correspond to all or a portion of a SECX protein. In one embodiment, a SECX fusion protein comprises at least one biologically active portion of a SECX protein. In another embodiment, a SECX fusion protein comprises at least two biologically active portions of a SECX protein. In yet another embodiment, a SECX fusion protein comprises at least three biologically active portions of a SECX protein. Within the fusion protein, the 35 term "operatively linked" is intended to indicate that the SECX polypeptide and the non-SECX

polypeptide are fused in-frame to each other. The non-SECX polypeptide can be fused to the N-terminus or C-terminus of the SECX polypeptide.

For example, in one embodiment a SECX fusion protein comprises a SECX domain operably linked to the extracellular domain of a second protein known to be involved in an activity of interest.

5 Such fusion proteins can be further utilized in screening assays for compounds which modulate SECX activity (such assays are described in detail below).

In one embodiment, the fusion protein is a GST-SECX fusion protein in which the SECX sequences are fused to the C-terminus of the GST (i.e., glutathione S-transferase) sequences. Such fusion proteins can facilitate the purification of recombinant SECX.

10 In another embodiment, the fusion protein is a SECX protein containing a heterologous signal sequence at its N-terminus. For example, the native SECX signal sequence (*i.e.*, about amino acids 1 to 26, or as described in Sections 1-14 above) can be removed and replaced with a signal sequence from another protein. In certain host cells (*e.g.*, mammalian host cells), expression and/or secretion of SECX can be increased through use of a heterologous signal sequence.

15 In yet another embodiment, the fusion protein is a SECX-immunoglobulin fusion protein in which the SECX sequences comprising primarily the extracellular domains are fused to sequences derived from a member of the immunoglobulin protein family. The SECX-immunoglobulin fusion proteins of the invention can be incorporated into pharmaceutical compositions and administered to a subject to inhibit an interaction between a SECX ligand and a SECX protein on the surface of a cell, to thereby suppress SECX-mediated signal transduction *in vivo*. The SECX-immunoglobulin fusion proteins can be used to affect the bioavailability of a SECX cognate ligand. Inhibition of the SECX ligand/SECX interaction may be useful therapeutically for both the treatment of proliferative and differentiative disorders, as well as modulating (*e.g.* promoting or inhibiting) cell survival. Moreover, the SECX-immunoglobulin fusion proteins of the invention can be used as immunogens to produce 20 anti-SECX antibodies in a subject, to purify SECX ligands, and in screening assays to identify molecules that inhibit the interaction of SECX with a SECX ligand.

25 A SECX chimeric or fusion protein of the invention can be produced by standard recombinant DNA techniques. For example, DNA fragments coding for the different polypeptide sequences are ligated together in-frame in accordance with conventional techniques, *e.g.*, by employing blunt-ended or stagger-ended termini for ligation, restriction enzyme digestion to provide for appropriate termini, filling-in of cohesive ends as appropriate, alkaline phosphatase treatment to avoid undesirable joining, and enzymatic ligation. In another embodiment, the fusion gene can be synthesized by conventional techniques including automated DNA synthesizers. Alternatively, PCR amplification of gene fragments can be carried out using anchor primers that give rise to complementary overhangs between 30 two consecutive gene fragments that can subsequently be annealed and reamplified to generate a chimeric gene sequence (see, for example, Ausubel *et al.* (eds.) CURRENT PROTOCOLS IN MOLECULAR

BIOLOGY, John Wiley & Sons, 1992). Moreover, many expression vectors are commercially available that already encode a fusion moiety (e.g., a GST polypeptide). A SECX-encoding nucleic acid can be cloned into such an expression vector such that the fusion moiety is linked in-frame to the SECX protein.

- 5 The invention also provides signal sequences derived from various SECX polypeptides. The signal sequences include, e.g., polypeptides including the signal peptides identified for the SECX polypeptides as predicted by the SignalP software program for the SECX polypeptides described above. These signal sequences are useful for directing a linked polypeptide sequence to a desired intracellular or extracellular (if secretion from the cell is desired) location. In some embodiments, the
10 signal sequence includes a portion of a SECX signal sequence that is sufficient to direct a linked polypeptide to a desired cellular compartment.

SECX agonists and antagonists

The present invention also pertains to variants of the SECX proteins that function as either SECX agonists (mimetics) or as SECX antagonists. Variants of the SECX protein can be generated by
15 mutagenesis, e.g., discrete point mutation or truncation of the SECX protein. An agonist of the SECX protein can retain substantially the same, or a subset of, the biological activities of the naturally occurring form of the SECX protein. An antagonist of the SECX protein can inhibit one or more of the activities of the naturally occurring form of the SECX protein by, for example, competitively binding to a downstream or upstream member of a cellular signaling cascade which includes the SECX
20 protein. Thus, specific biological effects can be elicited by treatment with a variant of limited function. In one embodiment, treatment of a subject with a variant having a subset of the biological activities of the naturally occurring form of the protein has fewer side effects in a subject relative to treatment with the naturally occurring form of the SECX proteins.

Variants of the SECX protein that function as either SECX agonists (mimetics) or as SECX
25 antagonists can be identified by screening combinatorial libraries of mutants, e.g., truncation mutants, of the SECX protein for SECX protein agonist or antagonist activity. In one embodiment, a variegated library of SECX variants is generated by combinatorial mutagenesis at the nucleic acid level and is encoded by a variegated gene library. A variegated library of SECX variants can be produced by, for example, enzymatically ligating a mixture of synthetic oligonucleotides into gene sequences such that
30 a degenerate set of potential SECX sequences is expressible as individual polypeptides, or alternatively, as a set of larger fusion proteins (e.g., for phage display) containing the set of SECX sequences therein. There are a variety of methods which can be used to produce libraries of potential SECX variants from a degenerate oligonucleotide sequence. Chemical synthesis of a degenerate gene sequence can be performed in an automatic DNA synthesizer, and the synthetic gene then ligated into
35 an appropriate expression vector. Use of a degenerate set of genes allows for the provision, in one

mixture, of all of the sequences encoding the desired set of potential SECX sequences. Methods for synthesizing degenerate oligonucleotides are known in the art (see, e.g., Narang (1983) *Tetrahedron* 39:3; Itakura *et al.* (1984) *Annu Rev Biochem* 53:323; Itakura *et al.* (1984) *Science* 198:1056; Ike *et al.* (1983) *Nucl Acid Res* 11:477.

5 **Polypeptide libraries**

In addition, libraries of fragments of the SECX protein coding sequence can be used to generate a variegated population of SECX fragments for screening and subsequent selection of variants of a SECX protein. In one embodiment, a library of coding sequence fragments can be generated by treating a double stranded PCR fragment of a SECX coding sequence with a nuclease 10 under conditions wherein nicking occurs only about once per molecule, denaturing the double stranded DNA, renaturing the DNA to form double stranded DNA that can include sense/antisense pairs from different nicked products, removing single stranded portions from reformed duplexes by treatment with S1 nuclease, and ligating the resulting fragment library into an expression vector. By this method, an expression library can be derived which encodes N-terminal and internal fragments of various sizes 15 of the SECX protein.

Several techniques are known in the art for screening gene products of combinatorial libraries made by point mutations or truncation, and for screening cDNA libraries for gene products having a selected property. Such techniques are adaptable for rapid screening of the gene libraries generated by the combinatorial mutagenesis of SECX proteins. The most widely used techniques, which are 20 amenable to high throughput analysis, for screening large gene libraries typically include cloning the gene library into replicable expression vectors, transforming appropriate cells with the resulting library of vectors, and expressing the combinatorial genes under conditions in which detection of a desired activity facilitates isolation of the vector encoding the gene whose product was detected. Recursive ensemble mutagenesis (REM), a new technique that enhances the frequency of functional 25 mutants in the libraries, can be used in combination with the screening assays to identify SECX variants (Arkin and Yourvan (1992) *PNAS* 89:7811-7815; Delgrave *et al.* (1993) *Protein Engineering* 6:327-331).

In one embodiment, cell based assays can be exploited to analyze a variegated SECX library, e.g., a library of mutant SECX polypeptides. For example, a library of expression vectors can be 30 transfected into a cell line that ordinarily responds to a particular ligand or receptor in a SECX-dependent manner, e.g., through a signaling complex. The transfected cells are then contacted with the putative SECX interactant and the effect of expression of the mutant SECX on signaling by the signaling complex can be detected, e.g. by measuring a cellular activity or cell survival. Plasmid DNA can then be recovered from the cells which score for inhibition, or alternatively, potentiation of, 35 e.g., cytokine induction, and the individual clones further characterized.

Anti-SECX antibodies

The invention encompasses antibodies and antibody fragments, such as F_{ab} or $(F_{ab})_2$, that bind immunospecifically to any of the polypeptides of the invention.

An isolated SECX protein, or a portion or fragment thereof, can be used as an immunogen to generate antibodies that bind SECX using standard techniques for polyclonal and monoclonal antibody preparation. The full-length SECX protein can be used or, alternatively, the invention provides antigenic peptide fragments of SECX for use as immunogens. The antigenic peptide of SECX comprises at least 4 amino acid residues of the amino acid sequence shown in SEQ ID NOs:2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22, 24, 26, 28, 75, 77, 79 and 81, plus 30 and 32 and encompasses an epitope of SECX such that an antibody raised against the peptide forms a specific immune complex with SECX. Preferably, the antigenic peptide comprises at least 6, 8, 10, 15, 20, or 30 amino acid residues. Longer antigenic peptides are sometimes preferable over shorter antigenic peptides, depending on use and according to methods well known to someone skilled in the art.

In certain embodiments of the invention, at least one epitope encompassed by the antigenic peptide is a region of SECX that is located on the surface of the protein, e.g., a hydrophilic region. A hydrophobicity analysis of the human SECX protein sequence will indicate which regions of a SECX polypeptide are particularly hydrophilic and, therefore, are likely to encode surface residues useful for targeting antibody production. As a means for targeting antibody production, hydropathy plots showing regions of hydrophilicity and hydrophobicity may be generated by any method well known in the art, including, for example, the Kyte Doolittle or the Hopp Woods methods, either with or without Fourier transformation. See, e.g., Hopp and Woods, 1981, *Proc. Nat. Acad. Sci. USA* 78: 3824-3828; Kyte and Doolittle 1982, *J. Mol. Biol.* 157: 105-142, each incorporated herein by reference in their entirety.

As disclosed herein, SECX protein sequence of SEQ ID NOs:2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22, 24, 26, 28, 75, 77, 79 and 81, plus 30 and 32, or derivatives, fragments, analogs or homologs thereof, may be utilized as immunogens in the generation of antibodies that immunospecifically-bind these protein components. The term "antibody" as used herein refers to immunoglobulin molecules and immunologically active portions of immunoglobulin molecules, i.e., molecules that contain an antigen binding site that specifically binds (immunoreacts with) an antigen, such as SECX. Such antibodies include, but are not limited to, polyclonal, monoclonal, chimeric, single chain, F_{ab} and $F_{(ab)}_2$ fragments, and an F_{ab} expression library. In a specific embodiment, antibodies to human SECX proteins are disclosed. Various procedures known within the art may be used for the production of polyclonal or monoclonal antibodies to a SECX protein sequence, or derivative, fragment, analog or homolog thereof. Some of these proteins are discussed below.

For the production of polyclonal antibodies, various suitable host animals (e.g., rabbit, goat, mouse or other mammal) may be immunized by injection with the native protein, or a synthetic

variant thereof, or a derivative of the foregoing. An appropriate immunogenic preparation can contain, for example, recombinantly expressed SECX protein or a chemically synthesized SECX polypeptide. The preparation can further include an adjuvant. Various adjuvants used to increase the immunological response include, but are not limited to, Freund's (complete and incomplete), mineral gels (e.g., aluminum hydroxide), surface active substances (e.g., lysolecithin, pluronic polyols, 5 polyanions, peptides, oil emulsions, dinitrophenol, etc.), human adjuvants such as *Bacille Calmette-Guerin* and *Corynebacterium parvum*, or similar immunostimulatory agents. If desired, the antibody molecules directed against SECX can be isolated from the mammal (e.g., from the blood) and further purified by well known techniques, such as protein A chromatography to obtain the IgG 10 fraction.

The term "monoclonal antibody" or "monoclonal antibody composition", as used herein, refers to a population of antibody molecules that contain only one species of an antigen binding site capable of immunoreacting with a particular epitope of SECX. A monoclonal antibody composition thus typically displays a single binding affinity for a particular SECX protein with which it immunoreacts. 15 For preparation of monoclonal antibodies directed towards a particular SECX protein, or derivatives, fragments, analogs or homologs thereof, any technique that provides for the production of antibody molecules by continuous cell line culture may be utilized. Such techniques include, but are not limited to, the hybridoma technique (see Kohler & Milstein, 1975 *Nature* 256: 495-497); the trioma technique; the human B-cell hybridoma technique (see Kozbor, *et al.*, 1983 *Immunol Today* 4: 72) and 20 the EBV hybridoma technique to produce human monoclonal antibodies (see Cole, *et al.*, 1985 In: MONOCLONAL ANTIBODIES AND CANCER THERAPY, Alan R. Liss, Inc., pp. 77-96). Human monoclonal antibodies may be utilized in the practice of the present invention and may be produced by using human hybridomas (see Cote, *et al.*, 1983. *Proc Natl Acad Sci USA* 80: 2026-2030) or by transforming human B-cells with Epstein Barr Virus *in vitro* (see Cole, *et al.*, 1985 In: MONOCLONAL 25 ANTIBODIES AND CANCER THERAPY, Alan R. Liss, Inc., pp. 77-96). Each of the above citations are incorporated herein by reference in their entirety.

According to the invention, techniques can be adapted for the production of single-chain antibodies specific to a SECX protein (see e.g., U.S. Patent No. 4,946,778). In addition, methodologies can be adapted for the construction of F_{ab} expression libraries (see e.g., Huse, *et al.*, 1989 *Science* 246: 30 1275-1281) to allow rapid and effective identification of monoclonal F_{ab} fragments with the desired specificity for a SECX protein or derivatives, fragments, analogs or homologs thereof. Non-human antibodies can be "humanized" by techniques well known in the art. See e.g., U.S. Patent No. 5,225,539. Antibody fragments that contain the idiotypes to a SECX protein may be produced by techniques known in the art including, but not limited to: (i) an F_{(ab')2} fragment produced by pepsin 35 digestion of an antibody molecule; (ii) an F_{ab} fragment generated by reducing the disulfide bridges of

an $F_{(ab')2}$ fragment; (iii) an F_{ab} fragment generated by the treatment of the antibody molecule with papain and a reducing agent and (iv) F_v fragments.

Additionally, recombinant anti-SECX antibodies, such as chimeric and humanized monoclonal antibodies, comprising both human and non-human portions, which can be made using standard recombinant DNA techniques, are within the scope of the invention. Such chimeric and humanized monoclonal antibodies can be produced by recombinant DNA techniques known in the art, for example using methods described in International Application No. PCT/US86/02269; European Patent Application No. 184,187; European Patent Application No. 171,496; European Patent Application No. 173,494; PCT International Publication No. WO 86/01533; U.S. Pat. No. 4,816,567; U.S. Pat. No. 5,225,539; European Patent Application No. 125,023; Better *et al.* (1988) *Science* 240:1041-1043; Liu *et al.* (1987) *PNAS* 84:3439-3443; Liu *et al.* (1987) *J Immunol.* 139:3521-3526; Sun *et al.* (1987) *PNAS* 84:214-218; Nishimura *et al.* (1987) *Cancer Res* 47:999-1005; Wood *et al.* (1985) *Nature* 314:446-449; Shaw *et al.* (1988) *J Natl Cancer Inst* 80:1553-1559; Morrison (1985) *Science* 229:1202-1207; Oi *et al.* (1986) *BioTechniques* 4:214; Jones *et al.* (1986) *Nature* 321:552-525; Verhoeven *et al.* (1988) *Science* 239:1534; and Beidler *et al.* (1988) *J Immunol* 141:4053-4060. Each of the above citations are incorporated herein by reference in their entirety.

In one embodiment, methodologies for the screening of antibodies that possess the desired specificity include, but are not limited to, enzyme-linked immunosorbent assay (ELISA) and other immunologically-mediated techniques known within the art. In a specific embodiment, selection of antibodies that are specific to a particular domain of a SECX protein is facilitated by generation of hybridomas that bind to the fragment of a SECX protein possessing such a domain. Antibodies that are specific for an above-described domain within a SECX protein, or derivatives, fragments, analogs or homologs thereof, are also provided herein.

Anti-SECX antibodies may be used in methods known within the art relating to the localization and/or quantitation of a SECX protein (e.g., for use in measuring levels of the SECX protein within appropriate physiological samples, for use in diagnostic methods, for use in imaging the protein, and the like). In a given embodiment, antibodies for SECX proteins, or derivatives, fragments, analogs or homologs thereof, that contain the antibody derived binding domain, are utilized as pharmacologically-active compounds [hereinafter "Therapeutics"].

An anti-SECX antibody (e.g., monoclonal antibody) can be used to isolate SECX by standard techniques, such as affinity chromatography or immunoprecipitation. An anti-SECX antibody can facilitate the purification of natural SECX from cells and of recombinantly produced SECX expressed in host cells. Moreover, an anti-SECX antibody can be used to detect SECX protein (e.g., in a cellular lysate or cell supernatant) in order to evaluate the abundance and pattern of expression of the SECX protein. Anti-SECX antibodies can be used diagnostically to monitor protein levels in tissue as part of a clinical testing procedure, e.g., to, for example, determine the efficacy of a given treatment regimen.

Detection can be facilitated by coupling (*i.e.*, physically linking) the antibody to a detectable substance. Examples of detectable substances include various enzymes, prosthetic groups, fluorescent materials, luminescent materials, bioluminescent materials, and radioactive materials. Examples of suitable enzymes include horseradish peroxidase, alkaline phosphatase, β -galactosidase, or 5 acetylcholinesterase; examples of suitable prosthetic group complexes include streptavidin/biotin and avidin/biotin; examples of suitable fluorescent materials include umbelliferone, fluorescein, fluorescein isothiocyanate, rhodamine, dichlorotriazinylamine fluorescein, dansyl chloride or phycoerythrin; an example of a luminescent material includes luminol; examples of bioluminescent materials include luciferase, luciferin, and aequorin, and examples of suitable radioactive material 10 include ^{125}I , ^{131}I , ^{35}S or ^3H .

SECX Recombinant Expression Vectors and Host Cells

Another aspect of the invention pertains to vectors, preferably expression vectors, containing a nucleic acid encoding SECX protein, or derivatives, fragments, analogs or homologs thereof. As used herein, the term "vector" refers to a nucleic acid molecule capable of transporting another nucleic acid to which it has been linked. One type of vector is a "plasmid", which refers to a circular double stranded DNA loop into which additional DNA segments can be ligated. Another type of vector is a viral vector, wherein additional DNA segments can be ligated into the viral genome. Certain vectors are capable of autonomous replication in a host cell into which they are introduced (*e.g.*, bacterial vectors having a bacterial origin of replication and episomal mammalian vectors). Other vectors (*e.g.*, 15 non-episomal mammalian vectors) are integrated into the genome of a host cell upon introduction into the host cell, and thereby are replicated along with the host genome. Moreover, certain vectors are capable of directing the expression of genes to which they are operatively linked. Such vectors are referred to herein as "expression vectors". In general, expression vectors of utility in recombinant DNA techniques are often in the form of plasmids. In the present specification, "plasmid" and "vector" 20 can be used interchangeably as the plasmid is the most commonly used form of vector. However, the invention is intended to include such other forms of expression vectors, such as viral vectors (*e.g.*, replication defective retroviruses, adenoviruses and adeno-associated viruses), which serve equivalent functions.

The recombinant expression vectors of the invention comprise a nucleic acid of the invention 25 in a form suitable for expression of the nucleic acid in a host cell, which means that the recombinant expression vectors include one or more regulatory sequences, selected on the basis of the host cells to be used for expression, that is operatively linked to the nucleic acid sequence to be expressed. Within a recombinant expression vector, "operably linked" is intended to mean that the nucleotide sequence of interest is linked to the regulatory sequence(s) in a manner that allows for expression of the 30 nucleotide sequence (*e.g.*, in an *in vitro* transcription/translation system or in a host cell when the 35

vector is introduced into the host cell). The term "regulatory sequence" is intended to include promoters, enhancers and other expression control elements (e.g., polyadenylation signals). Such regulatory sequences are described, for example, in Goeddel; GENE EXPRESSION TECHNOLOGY: METHODS IN ENZYMOLOGY 185, Academic Press, San Diego, Calif. (1990). Regulatory sequences 5 include those that direct constitutive expression of a nucleotide sequence in many types of host cell and those that direct expression of the nucleotide sequence only in certain host cells (e.g., tissue-specific regulatory sequences). It will be appreciated by those skilled in the art that the design of the expression vector can depend on such factors as the choice of the host cell to be transformed, the level of expression of protein desired, etc. The expression vectors of the invention can be 10 introduced into host cells to thereby produce proteins or peptides, including fusion proteins or peptides, encoded by nucleic acids as described herein (e.g., SECX proteins, mutant forms of SECX, fusion proteins, etc.).

The recombinant expression vectors of the invention can be designed for expression of SECX in prokaryotic or eukaryotic cells. For example, SECX can be expressed in bacterial cells such as *E. coli*, insect cells (using baculovirus expression vectors) yeast cells or mammalian cells. Suitable host 15 cells are discussed further in Goeddel, GENE EXPRESSION TECHNOLOGY: METHODS IN ENZYMOLOGY 185, Academic Press, San Diego, Calif. (1990). Alternatively, the recombinant expression vector can be transcribed and translated *in vitro*, for example using T7 promoter regulatory sequences and T7 polymerase.

20 Expression of proteins in prokaryotes is most often carried out in *E. coli* with vectors containing constitutive or inducible promoters directing the expression of either fusion or non-fusion proteins. Fusion vectors add a number of amino acids to a protein encoded therein, usually to the amino terminus of the recombinant protein. Such fusion vectors typically serve three purposes: (1) to increase expression of recombinant protein; (2) to increase the solubility of the recombinant protein; 25 and (3) to aid in the purification of the recombinant protein by acting as a ligand in affinity purification. Often, in fusion expression vectors, a proteolytic cleavage site is introduced at the junction of the fusion moiety and the recombinant protein to enable separation of the recombinant protein from the fusion moiety subsequent to purification of the fusion protein. Such enzymes, and their cognate recognition sequences, include Factor Xa, thrombin and enterokinase. Typical fusion 30 expression vectors include pGEX (Pharmacia Biotech Inc.; Smith and Johnson (1988) *Gene* 67:31-40), pMAL (New England Biolabs, Beverly, Mass.) and pRIT5 (Pharmacia, Piscataway, N.J.) that fuse glutathione S-transferase (GST), maltose E binding protein, or protein A, respectively, to the target recombinant protein.

35 Examples of suitable inducible non-fusion *E. coli* expression vectors include pTrc (Amrann *et al.*, (1988) *Gene* 69:301-315) and pET 11d (Studier *et al.*, GENE EXPRESSION TECHNOLOGY: METHODS IN ENZYMOLOGY 185, Academic Press, San Diego, Calif. (1990) 60-89).

One strategy to maximize recombinant protein expression in *E. coli* is to express the protein in a host bacteria with an impaired capacity to proteolytically cleave the recombinant protein. See, Gottesman, GENE EXPRESSION TECHNOLOGY: METHODS IN ENZYMOLOGY 185, Academic Press, San Diego, Calif. (1990) 119-128. Another strategy is to alter the nucleic acid sequence of the nucleic acid 5 to be inserted into an expression vector so that the individual codons for each amino acid are those preferentially utilized in *E. coli* (Wada *et al.*, (1992) *Nucleic Acids Res.* 20:2111-2118). Such alteration of nucleic acid sequences of the invention can be carried out by standard DNA synthesis techniques.

In another embodiment, the SECX expression vector is a yeast expression vector. Examples of 10 vectors for expression in yeast *S. cerevisiae* include pYEpSec1 (Baldari, *et al.*, (1987) *EMBO J* 6:229-234), pMFa (Kurjan and Herskowitz, (1982) *Cell* 30:933-943), pJRY88 (Schultz *et al.*, (1987) *Gene* 54:113-123), pYES2 (Invitrogen Corporation, San Diego, Calif.), and picZ (InVitrogen Corp., San Diego, Calif.).

Alternatively, SECX can be expressed in insect cells using baculovirus expression vectors. 15 Baculovirus vectors available for expression of proteins in cultured insect cells (*e.g.*, SF9 cells) include the pAc series (Smith *et al.* (1983) *Mol Cell Biol* 3:2156-2165) and the pVL series (Lucklow and Summers (1989) *Virology* 170:31-39).

In yet another embodiment, a nucleic acid of the invention is expressed in mammalian cells using a mammalian expression vector. Examples of mammalian expression vectors include pCDM8 20 (Seed (1987) *Nature* 329:840) and pMT2PC (Kaufman *et al.* (1987) *EMBO J* 6: 187-195). When used in mammalian cells, the expression vector's control functions are often provided by viral regulatory elements. For example, commonly used promoters are derived from polyoma, Adenovirus 2, cytomegalovirus and Simian Virus 40. For other suitable expression systems for both prokaryotic and eukaryotic cells. See, *e.g.*, Chapters 16 and 17 of Sambrook *et al.*, MOLECULAR CLONING: A 25 LABORATORY MANUAL, 2nd ed., Cold Spring Harbor Laboratory, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y., 1989.

In another embodiment, the recombinant mammalian expression vector is capable of directing 30 expression of the nucleic acid preferentially in a particular cell type (*e.g.*, tissue-specific regulatory elements are used to express the nucleic acid). Tissue-specific regulatory elements are known in the art. Non-limiting examples of suitable tissue-specific promoters include the albumin promoter (liver-specific; Pinkert *et al.* (1987) *Genes Dev* 1:268-277), lymphoid-specific promoters (Calame and Eaton (1988) *Adv Immunol* 43:235-275), in particular promoters of T cell receptors (Winoto and Baltimore (1989) *EMBO J* 8:729-733) and immunoglobulins (Banerji *et al.* (1983) *Cell* 33:729-740; Queen and Baltimore (1983) *Cell* 33:741-748), neuron-specific promoters (*e.g.*, the neurofilament 35 promoter; Byrne and Ruddle (1989) *PNAS* 86:5473-5477), pancreas-specific promoters (Edlund *et al.* (1985) *Science* 230:912-916), and mammary gland-specific promoters (*e.g.*, milk whey promoter; U.S.

Pat. No. 4,873,316 and European Application Publication No. 264,166). Developmentally-regulated promoters are also encompassed, e.g., the murine hox promoters (Kessel and Gruss (1990) *Science* 249:374-379) and the α -fetoprotein promoter (Campes and Tilghman (1989) *Genes Dev* 3:537-546).

The invention further provides a recombinant expression vector comprising a DNA molecule of the invention cloned into the expression vector in an antisense orientation. That is, the DNA molecule is operatively linked to a regulatory sequence in a manner that allows for expression (by transcription of the DNA molecule) of an RNA molecule that is antisense to SECX mRNA.

Regulatory sequences operatively linked to a nucleic acid cloned in the antisense orientation can be chosen that direct the continuous expression of the antisense RNA molecule in a variety of cell types, for instance viral promoters and/or enhancers, or regulatory sequences can be chosen that direct constitutive, tissue specific or cell type specific expression of antisense RNA. The antisense expression vector can be in the form of a recombinant plasmid, phagemid or attenuated virus in which antisense nucleic acids are produced under the control of a high efficiency regulatory region, the activity of which can be determined by the cell type into which the vector is introduced. For a discussion of the regulation of gene expression using antisense genes see Weintraub *et al.*, "Antisense RNA as a molecular tool for genetic analysis," *Reviews--Trends in Genetics*, Vol. 1(1) 1986.

Another aspect of the invention pertains to host cells into which a recombinant expression vector of the invention has been introduced. The terms "host cell" and "recombinant host cell" are used interchangeably herein. It is understood that such terms refer not only to the particular subject cell but to the progeny or potential progeny of such a cell. Because certain modifications may occur in succeeding generations due to either mutation or environmental influences, such progeny may not, in fact, be identical to the parent cell, but are still included within the scope of the term as used herein.

A host cell can be any prokaryotic or eukaryotic cell. For example, SECX protein can be expressed in bacterial cells such as *E. coli*, insect cells, yeast or mammalian cells (such as Chinese hamster ovary cells (CHO) or COS cells). Other suitable host cells are known to those skilled in the art.

Vector DNA can be introduced into prokaryotic or eukaryotic cells via conventional transformation or transfection techniques. As used herein, the terms "transformation" and "transfection" are intended to refer to a variety of art-recognized techniques for introducing foreign nucleic acid (e.g., DNA) into a host cell, including calcium phosphate or calcium chloride co-precipitation, DEAE-dextran-mediated transfection, lipofection, or electroporation. Suitable methods for transforming or transfecting host cells can be found in Sambrook, *et al.* (MOLECULAR CLONING: A LABORATORY MANUAL. 2nd ed., Cold Spring Harbor Laboratory, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y., 1989), and other laboratory manuals.

For stable transfection of mammalian cells, it is known that, depending upon the expression vector and transfection technique used, only a small fraction of cells may integrate the foreign DNA

into their genome. In order to identify and select these integrants, a gene that encodes a selectable marker (*e.g.*, resistance to antibiotics) is generally introduced into the host cells along with the gene of interest. Various selectable markers include those that confer resistance to drugs, such as G418, hygromycin and methotrexate. Nucleic acid encoding a selectable marker can be introduced into a host 5 cell on the same vector as that encoding SECX or can be introduced on a separate vector. Cells stably transfected with the introduced nucleic acid can be identified by drug selection (*e.g.*, cells that have incorporated the selectable marker gene will survive, while the other cells die).

A host cell of the invention, such as a prokaryotic or eukaryotic host cell in culture, can be used to produce (*i.e.*, express) SECX protein. Accordingly, the invention further provides methods for 10 producing SECX protein using the host cells of the invention. In one embodiment, the method comprises culturing the host cell of invention (into which a recombinant expression vector encoding SECX has been introduced) in a suitable medium such that SECX protein is produced. In another embodiment, the method further comprises isolating SECX from the medium or the host cell.

Transgenic animals

The host cells of the invention can also be used to produce nonhuman transgenic animals. For 15 example, in one embodiment, a host cell of the invention is a fertilized oocyte or an embryonic stem cell into which SECX-coding sequences have been introduced. Such host cells can then be used to create non-human transgenic animals in which exogenous SECX sequences have been introduced into their genome or homologous recombinant animals in which endogenous SECX sequences have been 20 altered. Such animals are useful for studying the function and/or activity of SECX and for identifying and/or evaluating modulators of SECX activity. As used herein, a "transgenic animal" is a non-human animal, preferably a mammal, more preferably a rodent such as a rat or mouse, in which one or more of the cells of the animal includes a transgene. Other examples of transgenic animals include 25 non-human primates, sheep, dogs, cows, goats, chickens, amphibians, etc. A transgene is exogenous DNA that is integrated into the genome of a cell from which a transgenic animal develops and that remains in the genome of the mature animal, thereby directing the expression of an encoded gene product in one or more cell types or tissues of the transgenic animal. As used herein, a "homologous recombinant animal" is a non-human animal, preferably a mammal, more preferably a mouse, in 30 which an endogenous SECX gene has been altered by homologous recombination between the endogenous gene and an exogenous DNA molecule introduced into a cell of the animal, *e.g.*, an embryonic cell of the animal, prior to development of the animal.

A transgenic animal of the invention can be created by introducing SECX-encoding nucleic acid into the male pronuclei of a fertilized oocyte, *e.g.*, by microinjection, retroviral infection, and allowing the oocyte to develop in a pseudopregnant female foster animal. The human SECX cDNA 35 can be introduced as a transgene into the genome of a non-human animal. Alternatively, a nonhuman

homologue of the human SECX gene, such as a mouse SECX gene, can be isolated based on hybridization to the human SECX cDNA (described further above) and used as a transgene. Intronic sequences and polyadenylation signals can also be included in the transgene to increase the efficiency of expression of the transgene. A tissue-specific regulatory sequence(s) can be operably linked to the
5 SECX transgene to direct expression of SECX protein to particular cells. Methods for generating transgenic animals via embryo manipulation and microinjection, particularly animals such as mice, have become conventional in the art and are described, for example, in U.S. Pat. Nos. 4,736,866; 4,870,009; and 4,873,191; and Hogan 1986, In: MANIPULATING THE MOUSE EMBRYO, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y. Similar methods are used for production of other
10 transgenic animals. A transgenic founder animal can be identified based upon the presence of the SECX transgene in its genome and/or expression of SECX mRNA in tissues or cells of the animals. A transgenic founder animal can then be used to breed additional animals carrying the transgene. Moreover, transgenic animals carrying a transgene encoding SECX can further be bred to other transgenic animals carrying other transgenes.

15 To create a homologous recombinant animal, a vector is prepared which contains at least a portion of a SECX gene into which a deletion, addition or substitution has been introduced to thereby alter, *e.g.*, functionally disrupt, the SECX gene. The SECX gene can be a human gene (*e.g.*, the cDNA of SEQ ID NOS:1, 3, 5, 7, 9, 11, 13, 15, 17, 19, 21, 23, 25, 27, 74, 76, 78 and 80, plus 29 and 31), but more preferably, is a non-human homologue of a human SECX gene. For example, a mouse
20 homologue of human SECX gene of, *e.g.*, SEQ ID NO:29, can be used to construct a homologous recombination vector suitable for altering an endogenous SECX gene in the mouse genome. In one embodiment, the vector is designed such that, upon homologous recombination, the endogenous SECX gene is functionally disrupted (*i.e.*, no longer encodes a functional protein; also referred to as a "knock out" vector).

25 Alternatively, the vector can be designed such that, upon homologous recombination, the endogenous SECX gene is mutated or otherwise altered but still encodes functional protein (*e.g.*, the upstream regulatory region can be altered to thereby alter the expression of the endogenous SECX protein). In the homologous recombination vector, the altered portion of the SECX gene is flanked at its 5' and 3' ends by additional nucleic acid of the SECX gene to allow for homologous recombination
30 to occur between the exogenous SECX gene carried by the vector and an endogenous SECX gene in an embryonic stem cell. The additional flanking SECX nucleic acid is of sufficient length for successful homologous recombination with the endogenous gene. Typically, several kilobases of flanking DNA (both at the 5' and 3' ends) are included in the vector. See *e.g.*, Thomas *et al.* (1987) Cell 51:503 for a description of homologous recombination vectors. The vector is introduced into an
35 embryonic stem cell line (*e.g.*, by electroporation) and cells in which the introduced SECX gene has

homologously recombined with the endogenous SECX gene are selected (see e.g., Li *et al.* (1992) *Cell* 69:915).

The selected cells are then injected into a blastocyst of an animal (e.g., a mouse) to form aggregation chimeras. See e.g., Bradley 1987, In: TERATOCARCINOMAS AND EMBRYONIC STEM CELLS: A PRACTICAL APPROACH, Robertson, ed. IRL, Oxford, pp. 113-152. A chimeric embryo can then be implanted into a suitable pseudopregnant female foster animal and the embryo brought to term. Progeny harboring the homologously recombined DNA in their germ cells can be used to breed animals in which all cells of the animal contain the homologously recombined DNA by germline transmission of the transgene. Methods for constructing homologous recombination vectors and homologous recombinant animals are described further in Bradley (1991) *Curr Opin Biotechnol* 2:823-829; PCT International Publication Nos.: WO 90/11354; WO 91/01140; WO 92/0968; and WO 93/04169.

In another embodiment, transgenic non-humans animals can be produced that contain selected systems that allow for regulated expression of the transgene. One example of such a system is the cre/loxP recombinase system of bacteriophage P1. For a description of the cre/loxP recombinase system, see, e.g., Lakso *et al.* (1992) *PNAS* 89:6232-6236. Another example of a recombinase system is the FLP recombinase system of *Saccharomyces cerevisiae* (O'Gorman *et al.* (1991) *Science* 251:1351-1355. If a cre/loxP recombinase system is used to regulate expression of the transgene, animals containing transgenes encoding both the Cre recombinase and a selected protein are required. Such animals can be provided through the construction of "double" transgenic animals, e.g., by mating two transgenic animals, one containing a transgene encoding a selected protein and the other containing a transgene encoding a recombinase.

Clones of the non-human transgenic animals described herein can also be produced according to the methods described in Wilmut *et al.* (1997) *Nature* 385:810-813. In brief, a cell, e.g., a somatic cell, from the transgenic animal can be isolated and induced to exit the growth cycle and enter G₀ phase. The quiescent cell can then be fused, e.g., through the use of electrical pulses, to an enucleated oocyte from an animal of the same species from which the quiescent cell is isolated. The reconstructed oocyte is then cultured such that it develops to morula or blastocyst and then transferred to pseudopregnant female foster animal. The offspring borne of this female foster animal will be a clone of the animal from which the cell, e.g., the somatic cell, is isolated.

Pharmaceutical Compositions

The SECX nucleic acid molecules, SECX proteins, and anti-SECX antibodies (also referred to herein as "active compounds") of the invention, and derivatives, fragments, analogs and homologs thereof, can be incorporated into pharmaceutical compositions suitable for administration. Such compositions typically comprise the nucleic acid molecule, protein, or antibody and a

pharmaceutically acceptable carrier. As used herein, "pharmaceutically acceptable carrier" is intended to include any and all solvents, dispersion media, coatings, antibacterial and antifungal agents, isotonic and absorption delaying agents, and the like, compatible with pharmaceutical administration. Suitable carriers are described in the most recent edition of Remington's Pharmaceutical Sciences, a 5 standard reference text in the field, which is incorporated herein by reference. Preferred examples of such carriers or diluents include, but are not limited to, water, saline, finger's solutions, dextrose solution, and 5% human serum albumin. Liposomes and non-aqueous vehicles such as fixed oils may also be used. The use of such media and agents for pharmaceutically active substances is well known in the art. Except insofar as any conventional media or agent is incompatible with the active 10 compound, use thereof in the compositions is contemplated. Supplementary active compounds can also be incorporated into the compositions.

A pharmaceutical composition of the invention is formulated to be compatible with its intended route of administration. Examples of routes of administration include parenteral, e.g., intravenous, intradermal, subcutaneous, oral (e.g., inhalation), transdermal (topical), transmucosal, and 15 rectal administration. Solutions or suspensions used for parenteral, intradermal, or subcutaneous application can include the following components: a sterile diluent such as water for injection, saline solution, fixed oils, polyethylene glycols, glycerine, propylene glycol or other synthetic solvents; antibacterial agents such as benzyl alcohol or methyl parabens; antioxidants such as ascorbic acid or sodium bisulfite; chelating agents such as ethylenediaminetetraacetic acid; buffers such as acetates, 20 citrates or phosphates, and agents for the adjustment of tonicity such as sodium chloride or dextrose. The pH can be adjusted with acids or bases, such as hydrochloric acid or sodium hydroxide. The parenteral preparation can be enclosed in ampoules, disposable syringes or multiple dose vials made of glass or plastic.

Pharmaceutical compositions suitable for injectable use include sterile aqueous solutions 25 (where water soluble) or dispersions and sterile powders for the extemporaneous preparation of sterile injectable solutions or dispersion. For intravenous administration, suitable carriers include physiological saline, bacteriostatic water, Cremophor EL™ (BASF, Parsippany, N.J.) or phosphate buffered saline (PBS). In all cases, the composition must be sterile and should be fluid to the extent that easy syringeability exists. It must be stable under the conditions of manufacture and storage and 30 must be preserved against the contaminating action of microorganisms such as bacteria and fungi. The carrier can be a solvent or dispersion medium containing, for example, water, ethanol, polyol (for example, glycerol, propylene glycol, and liquid polyethylene glycol, and the like), and suitable mixtures thereof. The proper fluidity can be maintained, for example, by the use of a coating such as lecithin, by the maintenance of the required particle size in the case of dispersion and by the use of 35 surfactants. Prevention of the action of microorganisms can be achieved by various antibacterial and antifungal agents, for example, parabens, chlorobutanol, phenol, ascorbic acid, thimerosal, and the

like. In many cases, it will be preferable to include isotonic agents, for example, sugars, polyalcohols such as manitol, sorbitol, sodium chloride in the composition. Prolonged absorption of the injectable compositions can be brought about by including in the composition an agent which delays absorption, for example, aluminum monostearate and gelatin.

5 Sterile injectable solutions can be prepared by incorporating the active compound (e.g., a SECX protein or anti-SECX antibody) in the required amount in an appropriate solvent with one or a combination of ingredients enumerated above, as required, followed by filtered sterilization.

Generally, dispersions are prepared by incorporating the active compound into a sterile vehicle that contains a basic dispersion medium and the required other ingredients from those enumerated above.

10 In the case of sterile powders for the preparation of sterile injectable solutions, methods of preparation are vacuum drying and freeze-drying that yields a powder of the active ingredient plus any additional desired ingredient from a previously sterile-filtered solution thereof.

Oral compositions generally include an inert diluent or an edible carrier. They can be enclosed in gelatin capsules or compressed into tablets. For the purpose of oral therapeutic administration, the 15 active compound can be incorporated with excipients and used in the form of tablets, troches, or capsules. Oral compositions can also be prepared using a fluid carrier for use as a mouthwash, wherein the compound in the fluid carrier is applied orally and swished and expectorated or swallowed. Pharmaceutically compatible binding agents, and/or adjuvant materials can be included as part of the 20 composition. The tablets, pills, capsules, troches and the like can contain any of the following ingredients, or compounds of a similar nature: a binder such as microcrystalline cellulose, gum tragacanth or gelatin; an excipient such as starch or lactose, a disintegrating agent such as alginic acid, Primogel, or corn starch; a lubricant such as magnesium stearate or Sterotes; a glidant such as colloidal silicon dioxide; a sweetening agent such as sucrose or saccharin; or a flavoring agent such as peppermint, methyl salicylate, or orange flavoring.

25 For administration by inhalation, the compounds are delivered in the form of an aerosol spray from pressured container or dispenser which contains a suitable propellant, e.g., a gas such as carbon dioxide, or a nebulizer.

Systemic administration can also be by transmucosal or transdermal means. For transmucosal or transdermal administration, penetrants appropriate to the barrier to be permeated are used in the 30 formulation. Such penetrants are generally known in the art, and include, for example, for transmucosal administration, detergents, bile salts, and fusidic acid derivatives. Transmucosal administration can be accomplished through the use of nasal sprays or suppositories. For transdermal administration, the active compounds are formulated into ointments, salves, gels, or creams as generally known in the art.

35 The compounds can also be prepared in the form of suppositories (e.g., with conventional suppository bases such as cocoa butter and other glycerides) or retention enemas for rectal delivery.

In one embodiment, the active compounds are prepared with carriers that will protect the compound against rapid elimination from the body, such as a controlled release formulation, including implants and microencapsulated delivery systems. Biodegradable, biocompatible polymers can be used, such as ethylene vinyl acetate, polyanhydrides, polyglycolic acid, collagen, polyorthoesters, and 5 polylactic acid. Methods for preparation of such formulations will be apparent to those skilled in the art. The materials can also be obtained commercially from Alza Corporation and Nova Pharmaceuticals, Inc. Liposomal suspensions (including liposomes targeted to infected cells with monoclonal antibodies to viral antigens) can also be used as pharmaceutically acceptable carriers. These can be prepared according to methods known to those skilled in the art, for example, as 10 described in U.S. Pat. No. 4,522,811.

It is especially advantageous to formulate oral or parenteral compositions in dosage unit form for ease of administration and uniformity of dosage. Dosage unit form as used herein refers to physically discrete units suited as unitary dosages for the subject to be treated; each unit containing a predetermined quantity of active compound calculated to produce the desired therapeutic effect in 15 association with the required pharmaceutical carrier. The specification for the dosage unit forms of the invention are dictated by and directly dependent on the unique characteristics of the active compound and the particular therapeutic effect to be achieved, and the limitations inherent in the art of compounding such an active compound for the treatment of individuals.

The nucleic acid molecules of the invention can be inserted into vectors and used as gene 20 therapy vectors. Gene therapy vectors can be delivered to a subject by, for example, intravenous injection, local administration (see U.S. Pat. No. 5,328,470) or by stereotactic injection (see e.g., Chen *et al.* (1994) *PNAS* 91:3054-3057). The pharmaceutical preparation of the gene therapy vector can include the gene therapy vector in an acceptable diluent, or can comprise a slow release matrix in which the gene delivery vehicle is imbedded. Alternatively, where the complete gene delivery vector 25 can be produced intact from recombinant cells, e.g., retroviral vectors, the pharmaceutical preparation can include one or more cells that produce the gene delivery system.

The pharmaceutical compositions can be included in a container, pack, or dispenser together with instructions for administration.

Uses and Methods of the Invention

30 The nucleic acid molecules, proteins, protein homologues, and antibodies described herein that include extracellular and transmembrane domains and, therefore, can be used in one or more of the following methods: (a) screening assays; (b) detection assays (e.g., chromosomal mapping, tissue typing, forensic biology), (c) predictive medicine (e.g., diagnostic assays, prognostic assays, monitoring clinical trials, and pharmacogenomics); and (d) methods of treatment (e.g., therapeutic and prophylactic). A SECX protein interacting with other cellular proteins can thus be used to (i) modulate 35

that respective protein activity; (ii) regulate cellular proliferation; (iii) regulate cellular differentiation; and (iv) regulate cell survival.

The isolated nucleic acid molecules of the invention can be used to express SECX protein (e.g., via a recombinant expression vector in a host cell in gene therapy applications), to detect SECX mRNA (e.g., in a biological sample) or a genetic lesion in a SECX gene, and to modulate SECX activity, as described further below. In addition, the SECX proteins can be used to screen drugs or compounds that modulate the SECX activity or expression as well as to treat disorders characterized by insufficient or excessive production of SECX protein or production of SECX protein forms that have decreased or aberrant activity compared to SECX wild type protein (e.g. proliferative disorders such as cancer or preclampsia, or any disease or disorder described in Sections 1-14 above). In addition, the anti-SECX antibodies of the invention can be used to detect and isolate SECX proteins and modulate SECX activity.

This invention further pertains to novel agents identified by the above described screening assays and uses thereof for treatments as described herein.

15 Screening Assays

The invention provides a method (also referred to herein as a "screening assay") for identifying modulators, i.e., candidate or test compounds or agents (e.g., peptides, peptidomimetics, small molecules or other drugs) that bind to SECX proteins or have a stimulatory or inhibitory effect on, for example, SECX expression or SECX activity.

20 In one embodiment, the invention provides assays for screening candidate or test compounds which bind to or modulate the activity of the membrane-bound form of a SECX protein or polypeptide or biologically active portion thereof. The test compounds of the present invention can be obtained using any of the numerous approaches in combinatorial library methods known in the art, including: biological libraries; spatially addressable parallel solid phase or solution phase libraries; synthetic library methods requiring deconvolution; the "one-bead one-compound" library method; and synthetic library methods using affinity chromatography selection. The biological library approach is limited to peptide libraries, while the other four approaches are applicable to peptide, non-peptide oligomer or small molecule libraries of compounds (Lam (1997) *Anticancer Drug Des* 12:145).

25 Examples of methods for the synthesis of molecular libraries can be found in the art, for example in: DeWitt *et al.* (1993) *Proc Natl Acad Sci U.S.A.* 90:6909; Erb *et al.* (1994) *Proc Natl Acad Sci U.S.A.* 91:11422; Zuckermann *et al.* (1994) *J Med Chem* 37:2678; Cho *et al.* (1993) *Science* 261:1303; Carrell *et al.* (1994) *Angew Chem Int Ed Engl* 33:2059; Carell *et al.* (1994) *Angew Chem Int Ed Engl* 33:2061; and Gallop *et al.* (1994) *J Med Chem* 37:1233.

30 Libraries of compounds may be presented in solution (e.g., Houghten (1992) *BioTechniques* 13:412-421), or on beads (Lam (1991) *Nature* 354:82-84), on chips (Fodor (1993) *Nature*

364:555-556), bacteria (Ladner U.S. Pat. No. 5,223,409), spores (Ladner USP '409), plasmids (Cull *et al.* (1992) *Proc Natl Acad Sci USA* 89:1865-1869) or on phage (Scott and Smith (1990) *Science* 249:386-390; Devlin (1990) *Science* 249:404-406; Cwirla *et al.* (1990) *Proc Natl Acad Sci U.S.A.* 87:6378-6382; Felici (1991) *J Mol Biol* 222:301-310; Ladner above.).

5 In one embodiment, an assay is a cell-based assay in which a cell which expresses a membrane-bound form of SECX protein, or a biologically active portion thereof, on the cell surface is contacted with a test compound and the ability of the test compound to bind to a SECX protein determined. The cell, for example, can of mammalian origin or a yeast cell. Determining the ability of the test compound to bind to the SECX protein can be accomplished, for example, by coupling the test
10 compound with a radioisotope or enzymatic label such that binding of the test compound to the SECX protein or biologically active portion thereof can be determined by detecting the labeled compound in a complex. For example, test compounds can be labeled with ^{125}I , ^{35}S , ^{14}C , or ^3H , either directly or indirectly, and the radioisotope detected by direct counting of radioemission or by scintillation counting. Alternatively, test compounds can be enzymatically labeled with, for example, horseradish
15 peroxidase, alkaline phosphatase, or luciferase, and the enzymatic label detected by determination of conversion of an appropriate substrate to product. In one embodiment, the assay comprises contacting a cell which expresses a membrane-bound form of SECX protein, or a biologically active portion thereof, on the cell surface with a known compound which binds SECX to form an assay mixture, contacting the assay mixture with a test compound, and determining the ability of the test compound
20 to interact with a SECX protein, wherein determining the ability of the test compound to interact with a SECX protein comprises determining the ability of the test compound to preferentially bind to SECX or a biologically active portion thereof as compared to the known compound.

In another embodiment, an assay is a cell-based assay comprising contacting a cell expressing a membrane-bound form of SECX protein, or a biologically active portion thereof, on the cell surface
25 with a test compound and determining the ability of the test compound to modulate (*e.g.*, stimulate or inhibit) the activity of the SECX protein or biologically active portion thereof. Determining the ability of the test compound to modulate the activity of SECX or a biologically active portion thereof can be accomplished, for example, by determining the ability of the SECX protein to bind to or interact with a SECX target molecule. As used herein, a "target molecule" is a molecule with which a SECX protein
30 binds or interacts in nature, for example, a molecule on the surface of a cell which expresses a SECX interacting protein, a molecule on the surface of a second cell, a molecule in the extracellular milieu, a molecule associated with the internal surface of a cell membrane or a cytoplasmic molecule. A SECX target molecule can be a non-SECX molecule or a SECX protein or polypeptide of the present invention. In one embodiment, a SECX target molecule is a component of a signal transduction
35 pathway that facilitates transduction of an extracellular signal (*e.g.* a signal generated by binding of a compound to a membrane-bound SECX molecule) through the cell membrane and into the cell. The

target, for example, can be a second intercellular protein that has catalytic activity or a protein that facilitates the association of downstream signaling molecules with SECX.

- Determining the ability of the SECX protein to bind to or interact with a SECX target molecule can be accomplished by one of the methods described above for determining direct binding.
- 5 In one embodiment, determining the ability of the SECX protein to bind to or interact with a SECX target molecule can be accomplished by determining the activity of the target molecule. For example, the activity of the target molecule can be determined by detecting induction of a cellular second messenger of the target (*i.e.* intracellular Ca^{2+} , diacylglycerol, IP_3 , etc.), detecting catalytic/enzymatic activity of the target an appropriate substrate, detecting the induction of a reporter gene (comprising a
- 10 SECX-responsive regulatory element operatively linked to a nucleic acid encoding a detectable marker, *e.g.*, luciferase), or detecting a cellular response, for example, cell survival, cellular differentiation, or cell proliferation.

In yet another embodiment, an assay of the present invention is a cell-free assay comprising contacting a SECX protein or biologically active portion thereof with a test compound and determining the ability of the test compound to bind to the SECX protein or biologically active portion thereof. Binding of the test compound to the SECX protein can be determined either directly or indirectly as described above. In one embodiment, the assay comprises contacting the SECX protein or biologically active portion thereof with a known compound which binds SECX to form an assay mixture, contacting the assay mixture with a test compound, and determining the ability of the test compound to interact with a SECX protein, wherein determining the ability of the test compound to interact with a SECX protein comprises determining the ability of the test compound to preferentially bind to SECX or biologically active portion thereof as compared to the known compound.

In another embodiment, an assay is a cell-free assay comprising contacting SECX protein or biologically active portion thereof with a test compound and determining the ability of the test compound to modulate (*e.g.* stimulate or inhibit) the activity of the SECX protein or biologically active portion thereof. Determining the ability of the test compound to modulate the activity of SECX can be accomplished, for example, by determining the ability of the SECX protein to bind to a SECX target molecule by one of the methods described above for determining direct binding. In an alternative embodiment, determining the ability of the test compound to modulate the activity of SECX can be accomplished by determining the ability of the SECX protein further modulate a SECX target molecule. For example, the catalytic/enzymatic activity of the target molecule on an appropriate substrate can be determined as previously described.

In yet another embodiment, the cell-free assay comprises contacting the SECX protein or biologically active portion thereof with a known compound which binds SECX to form an assay mixture, contacting the assay mixture with a test compound, and determining the ability of the test compound to interact with a SECX protein, wherein determining the ability of the test compound to

interact with a SECX protein comprises determining the ability of the SECX protein to preferentially bind to or modulate the activity of a SECX target molecule.

The cell-free assays of the present invention are amenable to use of both the soluble form or the membrane-bound form of SECX. In the case of cell-free assays comprising the membrane-bound form of SECX, it may be desirable to utilize a solubilizing agent such that the membrane-bound form of SECX is maintained in solution. Examples of such solubilizing agents include non-ionic detergents such as n-octylglucoside, n-dodecylglucoside, n-dodecylmaltoside, octanoyl-N-methylglucamide, decanoyl-N-methylglucamide, Triton® X-100, Triton® X-114, Thesit®, Isotridecypoly(ethylene glycol ether)_n, N-dodecyl-N,N-dimethyl-3-ammonio-1-propane sulfonate,

3-(3-cholamidopropyl)dimethylamminiol-1-propane sulfonate (CHAPS), or
3-(3-cholamidopropyl)dimethylamminiol-2-hydroxy-1-propane sulfonate (CHAPSO).

In more than one embodiment of the above assay methods of the present invention, it may be desirable to immobilize either SECX or its target molecule to facilitate separation of complexed from uncomplexed forms of one or both of the proteins, as well as to accommodate automation of the assay.

15 Binding of a test compound to SECX, or interaction of SECX with a target molecule in the presence and absence of a candidate compound, can be accomplished in any vessel suitable for containing the reactants. Examples of such vessels include microtiter plates, test tubes, and micro-centrifuge tubes. In one embodiment, a fusion protein can be provided that adds a domain that allows one or both of the proteins to be bound to a matrix. For example, GST-SECX fusion proteins or GST-target fusion

20 proteins can be adsorbed onto glutathione sepharose beads (Sigma Chemical, St. Louis, MO) or glutathione derivatized microtiter plates, that are then combined with the test compound or the test compound and either the non-adsorbed target protein or SECX protein, and the mixture is incubated under conditions conducive to complex formation (e.g., at physiological conditions for salt and pH). Following incubation, the beads or microtiter plate wells are washed to remove any unbound

25 components, the matrix immobilized in the case of beads, complex determined either directly or indirectly, for example, as described above. Alternatively, the complexes can be dissociated from the matrix, and the level of SECX binding or activity determined using standard techniques.

Other techniques for immobilizing proteins on matrices can also be used in the screening assays of the invention. For example, either SECX or its target molecule can be immobilized utilizing conjugation of biotin and streptavidin. Biotinylated SECX or target molecules can be prepared from biotin-NHS (N-hydroxy-succinimide) using techniques well known in the art (e.g., biotinylation kit, Pierce Chemicals, Rockford, Ill.), and immobilized in the wells of streptavidin-coated 96 well plates (Pierce Chemical). Alternatively, antibodies reactive with SECX or target molecules, but which do not interfere with binding of the SECX protein to its target molecule, can be derivatized to the wells of the plate, and unbound target or SECX trapped in the wells by antibody conjugation. Methods for detecting such complexes, in addition to those described above for the GST-immobilized complexes,

include immunodetection of complexes using antibodies reactive with the SECX or target molecule, as well as enzyme-linked assays that rely on detecting an enzymatic activity associated with the SECX or target molecule.

In another embodiment, modulators of SECX expression are identified in a method wherein a cell is contacted with a candidate compound and the expression of SECX mRNA or protein in the cell is determined. The level of expression of SECX mRNA or protein in the presence of the candidate compound is compared to the level of expression of SECX mRNA or protein in the absence of the candidate compound. The candidate compound can then be identified as a modulator of SECX expression based on this comparison. For example, when expression of SECX mRNA or protein is greater (statistically significantly greater) in the presence of the candidate compound than in its absence, the candidate compound is identified as a stimulator of SECX mRNA or protein expression. Alternatively, when expression of SECX mRNA or protein is less (statistically significantly less) in the presence of the candidate compound than in its absence, the candidate compound is identified as an inhibitor of SECX mRNA or protein expression. The level of SECX mRNA or protein expression in the cells can be determined by methods described herein for detecting SECX mRNA or protein.

In yet another aspect of the invention, the SECX proteins can be used as "bait proteins" in a two-hybrid assay or three hybrid assay (see, e.g., U.S. Pat. No. 5,283,317; Zervos *et al.* (1993) Cell 72:223-232; Madura *et al.* (1993) J Biol Chem 268:12046-12054; Bartel *et al.* (1993) BioTechniques 14:920-924; Iwabuchi *et al.* (1993) Oncogene 8:1693-1696; and Brent WO94/10300), to identify other proteins that bind to or interact with SECX ("SECX-binding proteins" or "SECX-bp") and modulate SECX activity. Such SECX-binding proteins are also likely to be involved in the propagation of signals by the SECX proteins as, for example, upstream or downstream elements of the SECX pathway.

The two-hybrid system is based on the modular nature of most transcription factors, which consist of separable DNA-binding and activation domains. Briefly, the assay utilizes two different DNA constructs. In one construct, the gene that codes for SECX is fused to a gene encoding the DNA binding domain of a known transcription factor (e.g., GAL-4). In the other construct, a DNA sequence, from a library of DNA sequences, that encodes an unidentified protein ("prey" or "sample") is fused to a gene that codes for the activation domain of the known transcription factor. If the "bait" and the "prey" proteins are able to interact, *in vivo*, forming a SECX-dependent complex, the DNA-binding and activation domains of the transcription factor are brought into close proximity. This proximity allows transcription of a reporter gene (e.g., LacZ) that is operably linked to a transcriptional regulatory site responsive to the transcription factor. Expression of the reporter gene can be detected and cell colonies containing the functional transcription factor can be isolated and used to obtain the cloned gene that encodes the protein which interacts with SECX.

This invention further pertains to novel agents identified by the above-described screening assays and uses thereof for treatments as described herein.

Detection Assays

Portions or fragments of the cDNA sequences identified herein (and the corresponding complete gene sequences) can be used in numerous ways as polynucleotide reagents. For example, these sequences can be used to: (i) map their respective genes on a chromosome; and, thus, locate gene regions associated with genetic disease; (ii) identify an individual from a minute biological sample (tissue typing); and (iii) aid in forensic identification of a biological sample. These applications are described in the subsections below.

10 Chromosome Mapping

Once the sequence (or a portion of the sequence) of a gene has been isolated, this sequence can be used to map the location of the gene on a chromosome. This process is called chromosome mapping. Accordingly, portions or fragments of the SECX sequences, described herein, can be used to map the location of the SECX genes, respectively, on a chromosome. The mapping of the SECX sequences to chromosomes is an important first step in correlating these sequences with genes associated with disease.

Briefly, SECX genes can be mapped to chromosomes by preparing PCR primers (preferably 15-25 bp in length) from the SECX sequences. Computer analysis of the SECX sequences can be used to rapidly select primers that do not span more than one exon in the genomic DNA, thus complicating the amplification process. These primers can then be used for PCR screening of somatic cell hybrids containing individual human chromosomes. Only those hybrids containing the human gene corresponding to the SECX sequences will yield an amplified fragment.

Somatic cell hybrids are prepared by fusing somatic cells from different mammals (e.g., human and mouse cells). As hybrids of human and mouse cells grow and divide, they gradually lose human chromosomes in random order, but retain the mouse chromosomes. By using media in which mouse cells cannot grow, because they lack a particular enzyme, but in which human cells can, the one human chromosome that contains the gene encoding the needed enzyme will be retained. By using various media, panels of hybrid cell lines can be established. Each cell line in a panel contains either a single human chromosome or a small number of human chromosomes, and a full set of mouse chromosomes, allowing easy mapping of individual genes to specific human chromosomes. (D'Eustachio *et al.* (1983) *Science* 220:919-924). Somatic cell hybrids containing only fragments of human chromosomes can also be produced by using human chromosomes with translocations and deletions.

35 PCR mapping of somatic cell hybrids is a rapid procedure for assigning a particular sequence to a particular chromosome. Three or more sequences can be assigned per day using a single thermal

cycler. Using the SECX sequences to design oligonucleotide primers, sublocalization can be achieved with panels of fragments from specific chromosomes.

Fluorescence *in situ* hybridization (FISH) of a DNA sequence to a metaphase chromosomal spread can further be used to provide a precise chromosomal location in one step. Chromosome spreads can be made using cells whose division has been blocked in metaphase by a chemical like colcemid that disrupts the mitotic spindle. The chromosomes can be treated briefly with trypsin, and then stained with Giemsa. A pattern of light and dark bands develops on each chromosome, so that the chromosomes can be identified individually. The FISH technique can be used with a DNA sequence as short as 500 or 600 bases. However, clones larger than 1,000 bases have a higher likelihood of binding to a unique chromosomal location with sufficient signal intensity for simple detection. Preferably 1,000 bases, and more preferably 2,000 bases, will suffice to get good results at a reasonable amount of time. For a review of this technique, see Verma *et al.*, HUMAN CHROMOSOMES: A MANUAL OF BASIC TECHNIQUES (Pergamon Press, New York 1988).

Reagents for chromosome mapping can be used individually to mark a single chromosome or a single site on that chromosome, or panels of reagents can be used for marking multiple sites and/or multiple chromosomes. Reagents corresponding to noncoding regions of the genes actually are preferred for mapping purposes. Coding sequences are more likely to be conserved within gene families, thus increasing the chance of cross hybridizations during chromosomal mapping.

Once a sequence has been mapped to a precise chromosomal location, the physical position of the sequence on the chromosome can be correlated with genetic map data. Such data are found, for example, in McKusick, MENDELIAN INHERITANCE IN MAN, available on-line through Johns Hopkins University Welch Medical Library). The relationship between genes and disease, mapped to the same chromosomal region, can then be identified through linkage analysis (co-inheritance of physically adjacent genes), described in, for example, Egeland *et al.* (1987) *Nature*, 325:783-787.

Moreover, differences in the DNA sequences between individuals affected and unaffected with a disease associated with the SECX gene, can be determined. If a mutation is observed in some or all of the affected individuals but not in any unaffected individuals, then the mutation is likely to be the causative agent of the particular disease. Comparison of affected and unaffected individuals generally involves first looking for structural alterations in the chromosomes, such as deletions or translocations that are visible from chromosome spreads or detectable using PCR based on that DNA sequence. Ultimately, complete sequencing of genes from several individuals can be performed to confirm the presence of a mutation and to distinguish mutations from polymorphisms.

Tissue Typing

The SECX sequences of the present invention can also be used to identify individuals from minute biological samples. In this technique, an individual's genomic DNA is digested with one or

more restriction enzymes, and probed on a Southern blot to yield unique bands for identification. The sequences of the present invention are useful as additional DNA markers for RFLP ("restriction fragment length polymorphisms," described in U.S. Pat. No. 5,272,057).

Furthermore, the sequences of the present invention can be used to provide an alternative
5 technique that determines the actual base-by-base DNA sequence of selected portions of an individual's genome. Thus, the SECX sequences described herein can be used to prepare two PCR primers from the 5' and 3' ends of the sequences. These primers can then be used to amplify an individual's DNA and subsequently sequence it.

Panels of corresponding DNA sequences from individuals, prepared in this manner, can
10 provide unique individual identifications, as each individual will have a unique set of such DNA sequences due to allelic differences. The sequences of the present invention can be used to obtain such identification sequences from individuals and from tissue. The SECX sequences of the invention uniquely represent portions of the human genome. Allelic variation occurs to some degree in the coding regions of these sequences, and to a greater degree in the noncoding regions. It is estimated
15 that allelic variation between individual humans occurs with a frequency of about once per each 500 bases. Much of the allelic variation is due to single nucleotide polymorphisms (SNPs), which include restriction fragment length polymorphisms (RFLPs).

Each of the sequences described herein can be used as a standard against which DNA from an individual can be compared for identification purposes. Because greater numbers of polymorphisms
20 occur in the noncoding regions, fewer sequences are necessary to differentiate individuals. The noncoding sequences of any one or more of SEQ ID NOs:1, 3, 5, 7, 9, 11, 13, 15, 17, 19, 21, 23, 25, 27, 74, 76, 78 and 80, plus 29 and 31 can comfortably provide positive individual identification with a panel of perhaps 10 to 1,000 primers that each yield a noncoding amplified sequence of 100 bases. If predicted coding sequences are used, a more appropriate number of primers for positive individual
25 identification would be 500-2,000.

A further use of the SECX sequences is to identify a cell or tissue type in a biological sample.
As discussed above, various SECX genes are expressed in one or more cell types. Thus, a cell type
30 can be identified based on the presence of RNA molecules from one or more SECX genes. Tissue distribution of various SECX genes are shown and discussed in FIGS. 19-23 and Examples 6-11, below.

Use of SECX Sequences in Forensic Biology

DNA-based identification techniques can also be used in forensic biology. Forensic biology is a scientific field employing genetic typing of biological evidence found at a crime scene as a means
35 for positively identifying, for example, a perpetrator of a crime. To make such an identification, PCR

technology can be used to amplify DNA sequences taken from very small biological samples such as tissues, *e.g.*, hair or skin, or body fluids, *e.g.*, blood, saliva, or semen found at a crime scene. The amplified sequence can then be compared to a standard, thereby allowing identification of the origin of the biological sample.

5 The sequences of the present invention can be used to provide polynucleotide reagents, *e.g.*, PCR primers, targeted to specific loci in the human genome, that can enhance the reliability of DNA-based forensic identifications by, for example, providing another "identification marker" (*i.e.* another DNA sequence that is unique to a particular individual). As mentioned above, actual base sequence information can be used for identification as an accurate alternative to patterns formed by
10 restriction enzyme generated fragments. Sequences targeted to noncoding regions of SECX gene are particularly appropriate for this use, as greater numbers of polymorphisms occur in the noncoding regions, making it easier to differentiate individuals using this technique. Examples of polynucleotide reagents include the SECX sequences or portions thereof, *e.g.*, fragments derived from the noncoding regions of a SECX gene described herein, having a length of at least 20 bases, preferably at least 30
15 bases.

The SECX sequences described herein can further be used to provide polynucleotide reagents, *e.g.*, labeled or labelable probes that can be used, for example, in an *in situ* hybridization technique, to identify a specific tissue, *e.g.*, brain tissue, etc. This can be very useful in cases where a forensic pathologist is presented with a tissue of unknown origin. Panels of such SECX probes can be used to
20 identify tissue by species and/or by organ type.

In a similar fashion, these reagents, *e.g.*, SECX primers or probes can be used to screen tissue culture for contamination (*i.e.* screen for the presence of a mixture of different types of cells in a culture).

Determination of the Biological Effect of the Therapeutic

25 In various embodiments of the present invention, suitable *in vitro* or *in vivo* assays are utilized to determine the effect of a specific Therapeutic and whether its administration is indicated for treatment of the affected tissue.

In various specific embodiments, *in vitro* assays may be performed with representative cells of the type(s) involved in the patient's disorder, to determine if a given Therapeutic exerts the desired
30 effect upon the cell type(s). Compounds for use in therapy may be tested in suitable animal model systems including, but not limited to rats, mice, chicken, cows, monkeys, rabbits, and the like, prior to testing in human subjects. Similarly, for *in vivo* testing, any of the animal model system known in the art may be used prior to administration to human subjects.

Malignancies

SECX proteins are located at the cellular membrane and are thought to be involved in the regulation of cell proliferation and differentiation. Accordingly, Therapeutics of the present invention may be useful in the therapeutic or prophylactic treatment of diseases or disorders that are associated with cell hyperproliferation and/or loss of control of cell proliferation (e.g., cancers, malignancies and tumors). For a review of such hyperproliferation disorders, see e.g., Fishman, *et al.*, 1985. MEDICINE, 2nd ed., J.B. Lippincott Co., Philadelphia, PA.

Therapeutics of the present invention may be assayed by any method known within the art for efficacy in treating or preventing malignancies and related disorders. Such assays include, but are not limited to, *in vitro* assays utilizing transformed cells or cells derived from the patient's tumor, as well as *in vivo* assays using animal models of cancer or malignancies. Potentially effective Therapeutics are those that, for example, inhibit the proliferation of tumor-derived or transformed cells in culture or cause a regression of tumors in animal models, in comparison to the controls.

In the practice of the present invention, once a malignancy or cancer has been shown to be amenable to treatment by modulating (*i.e.*, inhibiting, antagonizing or agonizing) activity, that cancer or malignancy may subsequently be treated or prevented by the administration of a Therapeutic that serves to modulate protein function.

Premalignant conditions

The Therapeutics of the present invention that are effective in the therapeutic or prophylactic treatment of cancer or malignancies may also be administered for the treatment of pre-malignant conditions and/or to prevent the progression of a pre-malignancy to a neoplastic or malignant state. Such prophylactic or therapeutic use is indicated in conditions known or suspected of preceding progression to neoplasia or cancer, in particular, where non-neoplastic cell growth consisting of hyperplasia, metaplasia or, most particularly, dysplasia has occurred. For a review of such abnormal cell growth see e.g., Robbins & Angell, 1976. BASIC PATHOLOGY, 2nd ed., W.B. Saunders Co., Philadelphia, PA.

Hyperplasia is a form of controlled cell proliferation involving an increase in cell number in a tissue or organ, without significant alteration in its structure or function. For example, it has been demonstrated that endometrial hyperplasia often precedes endometrial cancer. Metaplasia is a form of controlled cell growth in which one type of mature or fully differentiated cell substitutes for another type of mature cell. Metaplasia may occur in epithelial or connective tissue cells. Dysplasia is generally considered a precursor of cancer, and is found mainly in the epithelia. Dysplasia is the most disorderly form of non-neoplastic cell growth, and involves a loss in individual cell uniformity and in the architectural orientation of cells. Dysplasia characteristically occurs where there exists chronic

irritation or inflammation, and is often found in the cervix, respiratory passages, oral cavity, and gall bladder.

Alternatively, or in addition to the presence of abnormal cell growth characterized as hyperplasia, metaplasia, or dysplasia, the presence of one or more characteristics of a transformed or malignant phenotype displayed either *in vivo* or *in vitro* within a cell sample derived from a patient, is indicative of the desirability of prophylactic/therapeutic administration of a Therapeutic that possesses the ability to modulate activity of An aforementioned protein. Characteristics of a transformed phenotype include, but are not limited to: (i) morphological changes; (ii) looser substratum attachment; (iii) loss of cell-to-cell contact inhibition; (iv) loss of anchorage dependence; (v) protease release; (vi) increased sugar transport; (vii) decreased serum requirement; (viii) expression of fetal antigens, (ix) disappearance of the 250 kDa cell-surface protein, and the like. See e.g., Richards, *et al.*, 1986. MOLECULAR PATHOLOGY, W.B. Saunders Co., Philadelphia, PA.

In a specific embodiment of the present invention, a patient that exhibits one or more of the following predisposing factors for malignancy is treated by administration of an effective amount of a Therapeutic: (i) a chromosomal translocation associated with a malignancy (e.g., the Philadelphia chromosome (*bcr/abl*) for chronic myelogenous leukemia and t(14;18) for follicular lymphoma, etc.); (ii) familial polyposis or Gardner's syndrome (possible forerunners of colon cancer); (iii) monoclonal gammopathy of undetermined significance (a possible precursor of multiple myeloma) and (iv) a first degree kinship with persons having a cancer or pre-cancerous disease showing a Mendelian (genetic) inheritance pattern (e.g., familial polyposis of the colon, Gardner's syndrome, hereditary exostosis, polyendocrine adenomatosis, Peutz-Jeghers syndrome, neurofibromatosis of Von Recklinghausen, medullary thyroid carcinoma with amyloid production and pheochromocytoma, retinoblastoma, carotid body tumor, cutaneous melanocarcinoma, intraocular melanocarcinoma, xeroderma pigmentosum, ataxia telangiectasia, Chediak-Higashi syndrome, albinism, Fanconi's aplastic anemia and Bloom's syndrome).

In another embodiment, a Therapeutic of the present invention is administered to a human patient to prevent the progression to breast, colon, lung, pancreatic, or uterine cancer, or melanoma or sarcoma.

Hyperproliferative and dysproliferative disorders

In one embodiment of the present invention, a Therapeutic is administered in the therapeutic or prophylactic treatment of hyperproliferative or benign dysproliferative disorders. The efficacy in treating or preventing hyperproliferative diseases or disorders of a Therapeutic of the present invention may be assayed by any method known within the art. Such assays include *in vitro* cell proliferation assays, *in vitro* or *in vivo* assays using animal models of hyperproliferative diseases or

disorders, or the like. Potentially effective Therapeutics may, for example, promote cell proliferation in culture or cause growth or cell proliferation in animal models in comparison to controls.

Specific embodiments of the present invention are directed to the treatment or prevention of cirrhosis of the liver (a condition in which scarring has overtaken normal liver regeneration processes); treatment of keloid (hypertrophic scar) formation causing disfiguring of the skin in which the scarring process interferes with normal renewal; psoriasis (a common skin condition characterized by excessive proliferation of the skin and delay in proper cell fate determination); benign tumors; fibrocystic conditions and tissue hypertrophy (e.g., benign prostatic hypertrophy).

Neurodegenerative disorders

SECX protein have been implicated in the deregulation of cellular maturation and apoptosis, which are both characteristic of neurodegenerative disease. Accordingly, Therapeutics of the invention, particularly but not limited to those that modulate (or supply) activity of an aforementioned protein, may be effective in treating or preventing neurodegenerative disease. Therapeutics of the present invention that modulate the activity of an aforementioned protein involved in neurodegenerative disorders can be assayed by any method known in the art for efficacy in treating or preventing such neurodegenerative diseases and disorders. Such assays include *in vitro* assays for regulated cell maturation or inhibition of apoptosis or *in vivo* assays using animal models of neurodegenerative diseases or disorders, or any of the assays described below. Potentially effective Therapeutics, for example but not by way of limitation, promote regulated cell maturation and prevent cell apoptosis in culture, or reduce neurodegeneration in animal models in comparison to controls.

Once a neurodegenerative disease or disorder has been shown to be amenable to treatment by modulation activity, that neurodegenerative disease or disorder can be treated or prevented by administration of a Therapeutic that modulates activity. Such diseases include all degenerative disorders involved with aging, especially osteoarthritis and neurodegenerative disorders.

Disorders related to organ transplantation

SECX has been implicated in disorders related to organ transplantation, in particular but not limited to organ rejection. Therapeutics of the invention, particularly those that modulate (or supply) activity, may be effective in treating or preventing diseases or disorders related to organ transplantation. Therapeutics of the invention (particularly Therapeutics that modulate the levels or activity of an aforementioned protein) can be assayed by any method known in the art for efficacy in treating or preventing such diseases and disorders related to organ transplantation. Such assays include *in vitro* assays for using cell culture models as described below, or *in vivo* assays using animal models of diseases and disorders related to organ transplantation, see e.g., below. Potentially effective Therapeutics, for example but not by way of limitation, reduce immune rejection responses in animal models in comparison to controls.

Accordingly, once diseases and disorders related to organ transplantation are shown to be amenable to treatment by modulation of activity, such diseases or disorders can be treated or prevented by administration of a Therapeutic that modulates activity.

Cardiovascular Disease

5 SECX has been implicated in cardiovascular disorders, including in atherosclerotic plaque formation. Diseases such as cardiovascular disease, including cerebral thrombosis or hemorrhage, ischemic heart or renal disease, peripheral vascular disease, or thrombosis of other major vessel, and other diseases, including diabetes mellitus, hypertension, hypothyroidism, cholesterol ester storage disease, systemic lupus erythematosus, homocysteinemia, and familial protein or lipid processing 10 diseases, and the like, are either directly or indirectly associated with atherosclerosis. Accordingly, Therapeutics of the invention, particularly those that modulate (or supply) activity or formation may be effective in treating or preventing atherosclerosis-associated diseases or disorders. Therapeutics of the invention (particularly Therapeutics that modulate the levels or activity) can be assayed by any method known in the art, including those described below, for efficacy in treating or preventing such 15 diseases and disorders.

A vast array of animal and cell culture models exist for processes involved in atherosclerosis. A limited and non-exclusive list of animal models includes knockout mice for premature atherosclerosis (Kurabayashi and Yazaki, 1996, Int. Angiol. 15: 187-194), transgenic mouse models of atherosclerosis (Kappel *et al.*, 1994, FASEB J. 8: 583-592), antisense oligonucleotide treatment of 20 animal models (Callow, 1995, Curr. Opin. Cardiol. 10: 569-576), transgenic rabbit models for atherosclerosis (Taylor, 1997, Ann. N.Y. Acad. Sci 811: 146-152), hypercholesterolemic animal models (Rosenfeld, 1996, Diabetes Res. Clin. Pract. 30 Suppl.: 1-11), hyperlipidemic mice (Paigen *et al.*, 1994, Curr. Opin. Lipidol. 5: 258-264), and inhibition of lipoxygenase in animals (Sigal *et al.*, 1994, Ann. N.Y. Acad. Sci. 714: 211-224). In addition, *in vitro* cell models include but are not limited 25 to monocytes exposed to low density lipoprotein (Frostegard *et al.*, 1996, Atherosclerosis 121: 93-103), cloned vascular smooth muscle cells (Suttles *et al.*, 1995, Exp. Cell Res. 218: 331-338), endothelial cell-derived chemoattractant exposed T cells (Katz *et al.*, 1994, J. Leukoc. Biol. 55: 567-573), cultured human aortic endothelial cells (Farber *et al.*, 1992, Am. J. Physiol. 262: H1088-1085), and foam cell cultures (Libby *et al.*, 1996, Curr Opin Lipidol 7: 330-335). Potentially 30 effective Therapeutics, for example but not by way of limitation, reduce foam cell formation in cell culture models, or reduce atherosclerotic plaque formation in hypercholesterolemic mouse models of atherosclerosis in comparison to controls.

Accordingly, once an atherosclerosis-associated disease or disorder has been shown to be amenable to treatment by modulation of activity or formation, that disease or disorder can be treated 35 or prevented by administration of a Therapeutic that modulates activity.

Cytokine and Cell Proliferation/Differentiation Activity

A SECX protein of the present invention may exhibit cytokine, cell proliferation (either inducing or inhibiting) or cell differentiation (either inducing or inhibiting) activity or may induce production of other cytokines in certain cell populations. Many protein factors discovered to date, 5 including all known cytokines, have exhibited activity in one or more factor dependent cell proliferation assays, and hence the assays serve as a convenient confirmation of cytokine activity. The activity of a protein of the present invention is evidenced by any one of a number of routine factor dependent cell proliferation assays for cell lines including, without limitation, 32D, DA2, DA1G, T10, B9, B9/11, BaF3, MC9/G, M+ (preB M+), 2E8, RB5, DA1, 123, T1165, HT2, CTLL2, TF-1, Mo7e 10 and CMK.

The activity of a protein of the invention may, among other means, be measured by the following methods: Assays for T-cell or thymocyte proliferation include without limitation those described in: CURRENT PROTOCOLS IN IMMUNOLOGY, Ed by Coligan *et al.*, Greene Publishing Associates and Wiley-Interscience (Chapter 3 and Chapter 7); Takai *et al.*, *J Immunol* 137:3494-3500, 15 1986; Bertagnoli *et al.*, *J Immunol* 145:1706-1712, 1990; Bertagnolli *et al.*, *Cell Immunol* 133:327-341, 1991; Bertagnolli, *et al.*, *J Immunol* 149:3778-3783, 1992; Bowman *et al.*, *J Immunol* 152:1756-1761, 1994.

Assays for cytokine production and/or proliferation of spleen cells, lymph node cells or thymocytes include, without limitation, those described by Kruisbeek and Shevach, In: CURRENT 20 PROTOCOLS IN IMMUNOLOGY. Coligan *et al.*, eds. Vol 1, pp. 3.12.1-14, John Wiley and Sons, Toronto 1994; and by Schreiber, In: CURRENT PROTOCOLS IN IMMUNOLOGY. Coligan eds. Vol 1 pp. 6.8.1-8, John Wiley and Sons, Toronto 1994.

Assays for proliferation and differentiation of hematopoietic and lymphopoietic cells include, without limitation, those described by Bottomly *et al.*, In: CURRENT PROTOCOLS IN IMMUNOLOGY. 25 Coligan *et al.*, eds. Vol 1 pp. 6.3.1-6.3.12, John Wiley and Sons, Toronto 1991; deVries *et al.*, *J Exp Med* 173:1205-1211, 1991; Moreau *et al.*, *Nature* 336:690-692, 1988; Greenberger *et al.*, *Proc Natl Acad Sci U.S.A.* 80:2931-2938, 1983; Nordan, In: CURRENT PROTOCOLS IN IMMUNOLOGY. Coligan *et al.*, eds. Vol 1 pp. 6.6.1-5, John Wiley and Sons, Toronto 1991; Smith *et al.*, *Proc Natl Acad Sci U.S.A.* 83:1857-1861, 1986; Measurement of human Interleukin 11-Bennett, *et al.* In: CURRENT 30 PROTOCOLS IN IMMUNOLOGY. Coligan *et al.*, eds. Vol 1 pp. 6.15.1 John Wiley and Sons, Toronto 1991; Ciarletta, *et al.*, In: CURRENT PROTOCOLS IN IMMUNOLOGY. Coligan *et al.*, eds. Vol 1 pp. 6.13.1, John Wiley and Sons, Toronto 1991.

Assays for T-cell clone responses to antigens (which will identify, among others, proteins that affect APC-T cell interactions as well as direct T-cell effects by measuring proliferation and cytokine 35 production) include, without limitation, those described In: CURRENT PROTOCOLS IN IMMUNOLOGY. Coligan *et al.*, eds., Greene Publishing Associates and Wiley-Interscience (Chapter 3Chapter 6,

Chapter 7); Weinberger *et al.*, *Proc Natl Acad Sci USA* 77:6091-6095, 1980; Weinberger *et al.*, *Eur J Immun* 11:405-411, 1981; Takai *et al.*, *J Immunol* 137:3494-3500, 1986; Takai *et al.*, *J Immunol* 140:508-512, 1988.

Immune Stimulating or Suppressing Activity

5 A SECX protein of the present invention may also exhibit immune stimulating or immune suppressing activity, including without limitation the activities for which assays are described herein. A protein may be useful in the treatment of various immune deficiencies and disorders (including severe combined immunodeficiency (SCID)), *e.g.*, in regulating (up or down) growth and proliferation of T and/or B lymphocytes, as well as effecting the cytolytic activity of NK cells and other cell populations. These immune deficiencies may be genetic or be caused by viral (*e.g.*, HIV) as well as bacterial or fungal infections, or may result from autoimmune disorders. More specifically, infectious diseases causes by viral, bacterial, fungal or other infection may be treatable using a protein of the present invention, including infections by HIV, hepatitis viruses, herpesviruses, mycobacteria, Leishmania species., malaria species. and various fungal infections such as candidiasis. Of course, in 10 this regard, a protein of the present invention may also be useful where a boost to the immune system generally may be desirable, *i.e.*, in the treatment of cancer.

15

Autoimmune disorders which may be treated using a protein of the present invention include, for example, connective tissue disease, multiple sclerosis, systemic lupus erythematosus, rheumatoid arthritis, autoimmune pulmonary inflammation, Guillain-Barre syndrome, autoimmune thyroiditis, 20 insulin dependent diabetes mellitus, myasthenia gravis, graft-versus-host disease and autoimmune inflammatory eye disease. Such a protein of the present invention may also be useful in the treatment of allergic reactions and conditions, such as asthma (particularly allergic asthma) or other respiratory problems. Other conditions, in which immune suppression is desired (including, for example, organ transplantation), may also be treatable using a protein of the present invention.

25 Using the proteins of the invention it may also be possible to modulate immune responses, in a number of ways. Down regulation may be in the form of inhibiting or blocking an immune response already in progress or may involve preventing the induction of an immune response. The functions of activated T cells may be inhibited by suppressing T cell responses or by inducing specific tolerance in T cells, or both. Immunosuppression of T cell responses is generally an active, non-antigen-specific, process 30 which requires continuous exposure of the T cells to the suppressive agent. Tolerance, which involves inducing non-responsiveness or energy in T cells, is distinguishable from immunosuppression in that it is generally antigen-specific and persists after exposure to the tolerizing agent has ceased. Operationally, tolerance can be demonstrated by the lack of a T cell response upon re-exposure to specific antigen in the absence of the tolerizing agent.

Down regulating or preventing one or more antigen functions (including without limitation B lymphocyte antigen functions (such as, for example, B7), e.g., preventing high level lymphokine synthesis by activated T cells, will be useful in situations of tissue, skin and organ transplantation and in graft-versus-host disease (GVHD). For example, blockage of T cell function should result in

5 reduced tissue destruction in tissue transplantation. Typically, in tissue transplants, rejection of the transplant is initiated through its recognition as foreign by T cells, followed by an immune reaction that destroys the transplant. The administration of a molecule which inhibits or blocks interaction of a B7 lymphocyte antigen with its natural ligand(s) on immune cells (such as a soluble, monomeric form of a peptide having B7-2 activity alone or in conjunction with a monomeric form of a peptide having

10 an activity of another B lymphocyte antigen (e.g., B7-1, B7-3) or blocking antibody), prior to transplantation can lead to the binding of the molecule to the natural ligand(s) on the immune cells without transmitting the corresponding costimulatory signal. Blocking B lymphocyte antigen function in this matter prevents cytokine synthesis by immune cells, such as T cells, and thus acts as an immunosuppressant. Moreover, the lack of costimulation may also be sufficient to energize the T

15 cells, thereby inducing tolerance in a subject. Induction of long-term tolerance by B lymphocyte antigen-blocking reagents may avoid the necessity of repeated administration of these blocking reagents. To achieve sufficient immunosuppression or tolerance in a subject, it may also be necessary to block the function of B lymphocyte antigens.

The efficacy of particular blocking reagents in preventing organ transplant rejection or GVHD

20 can be assessed using animal models that are predictive of efficacy in humans. Examples of appropriate systems which can be used include allogeneic cardiac grafts in rats and xenogeneic pancreatic islet cell grafts in mice, both of which have been used to examine the immunosuppressive effects of CTLA4Ig fusion proteins *in vivo* as described in Lenschow *et al.*, Science 257:789-792 (1992) and Turka *et al.*, Proc Natl Acad Sci USA, 89:11102-11105 (1992). In addition, murine models

25 of GVHD (see Paul ed., FUNDAMENTAL IMMUNOLOGY, Raven Press, New York, 1989, pp. 846-847) can be used to determine the effect of blocking B lymphocyte antigen function *in vivo* on the development of that disease.

Blocking antigen function may also be therapeutically useful for treating autoimmune diseases. Many autoimmune disorders are the result of inappropriate activation of T cells that are

30 reactive against self tissue and which promote the production of cytokines and auto-antibodies involved in the pathology of the diseases. Preventing the activation of autoreactive T cells may reduce or eliminate disease symptoms. Administration of reagents which block costimulation of T cells by disrupting receptor:ligand interactions of B lymphocyte antigens can be used to inhibit T cell activation and prevent production of auto-antibodies or T cell-derived cytokines which may be

35 involved in the disease process. Additionally, blocking reagents may induce antigen-specific tolerance of autoreactive T cells which could lead to long-term relief from the disease. The efficacy of blocking

reagents in preventing or alleviating autoimmune disorders can be determined using a number of well-characterized animal models of human autoimmune diseases. Examples include murine experimental autoimmune encephalitis, systemic lupus erythematosus in MRL/lpr/lpr mice or NZB hybrid mice, murine autoimmune collagen arthritis, diabetes mellitus in NOD mice and BB rats, and 5 murine experimental myasthenia gravis (see Paul ed., FUNDAMENTAL IMMUNOLOGY, Raven Press, New York, 1989, pp. 840-856).

Upregulation of an antigen function (preferably a B lymphocyte antigen function), as a means of up regulating immune responses, may also be useful in therapy. Upregulation of immune responses may be in the form of enhancing an existing immune response or eliciting an initial immune response. 10 For example, enhancing an immune response through stimulating B lymphocyte antigen function may be useful in cases of viral infection. In addition, systemic vital diseases such as influenza, the common cold, and encephalitis might be alleviated by the administration of stimulatory forms of B lymphocyte antigens systemically.

Alternatively, anti-viral immune responses may be enhanced in an infected patient by 15 removing T cells from the patient, costimulating the T cells *in vitro* with viral antigen-pulsed APCs either expressing a peptide of the present invention or together with a stimulatory form of a soluble peptide of the present invention and reintroducing the *in vitro* activated T cells into the patient. Another method of enhancing anti-vital immune responses would be to isolate infected cells from a 20 patient, transflect them with a nucleic acid encoding a protein of the present invention as described herein such that the cells express all or a portion of the protein on their surface, and reintroduce the transfected cells into the patient. The infected cells would now be capable of delivering a costimulatory signal to, and thereby activate, T cells *in vivo*.

In another application, up regulation or enhancement of antigen function (preferably B 25 lymphocyte antigen function) may be useful in the induction of tumor immunity. Tumor cells (e.g., sarcoma, melanoma, lymphoma, leukemia, neuroblastoma, carcinoma) transfected with a nucleic acid encoding at least one peptide of the present invention can be administered to a subject to overcome tumor-specific tolerance in the subject. If desired, the tumor cell can be transfected to express a combination of peptides. For example, tumor cells obtained from a patient can be transfected *ex vivo* 30 with an expression vector directing the expression of a peptide having B7-2-like activity alone, or in conjunction with a peptide having B7-1-like activity and/or B7-3-like activity. The transfected tumor cells are returned to the patient to result in expression of the peptides on the surface of the transfected cell. Alternatively, gene therapy techniques can be used to target a tumor cell for transfection *in vivo*.

The presence of the peptide of the present invention having the activity of a B lymphocyte 35 antigen(s) on the surface of the tumor cell provides the necessary costimulation signal to T cells to induce a T cell mediated immune response against the transfected tumor cells. In addition, tumor cells which lack MHC class I or MHC class II molecules, or which fail to reexpress sufficient amounts of

MHC class I or MHC class II molecules, can be transfected with nucleic acid encoding all or a portion of (e.g., a cytoplasmic-domain truncated portion) of an MHC class I α chain protein and β_2 microglobulin protein or an MHC class II α chain protein and an MHC class II β chain protein to thereby express MHC class I or MHC class II proteins on the cell surface. Expression of the
5 appropriate class I or class II MHC in conjunction with a peptide having the activity of a B lymphocyte antigen (e.g., B7-1, B7-2, B7-3) induces a T cell mediated immune response against the transfected tumor cell. Optionally, a gene encoding an antisense construct which blocks expression of an MHC class II associated protein, such as the invariant chain, can also be cotransfected with a DNA encoding a peptide having the activity of a B lymphocyte antigen to promote presentation of tumor
10 associated antigens and induce tumor specific immunity. Thus, the induction of a T cell mediated immune response in a human subject may be sufficient to overcome tumor-specific tolerance in the subject.

The activity of a protein of the invention may, among other means, be measured by the following methods: Suitable assays for thymocyte or splenocyte cytotoxicity include, without
15 limitation, those described In: CURRENT PROTOCOLS IN IMMUNOLOGY. Coligan *et al.*, eds. Greene Publishing Associates and Wiley-Interscience (Chapter 3, Chapter 7); Herrmann *et al.*, *Proc Natl Acad Sci USA* 78:2488-2492, 1981; Herrmann *et al.*, *J Immunol* 128:1968-1974, 1982; Handa *et al.*, *J Immunol* 135:1564-1572, 1985; Takai *et al.*, *J Immunol* 137:3494-3500, 1986; Takai *et al.*, *J Immunol* 140:508-512, 1988; Herrmann *et al.*, *Proc Natl Acad Sci USA* 78:2488-2492, 1981; Herrmann *et al.*, *J Immunol* 128:1968-1974, 1982; Handa *et al.*, *J Immunol* 135:1564-1572, 1985; Takai *et al.*, *J Immunol* 137:3494-3500, 1986; Bowman *et al.*, *J Virology* 61:1992-1998; Takai *et al.*, *J Immunol* 140:508-512, 1988; Bertagnolli *et al.*, *Cell Immunol* 133:327-341, 1991; Brown *et al.*, *J Immunol* 153:3079-3092, 1994.

Assays for T-cell-dependent immunoglobulin responses and isotype switching (which will
25 identify, among others, proteins that modulate T-cell dependent antibody responses and that affect Th1/Th2 profiles) include, without limitation, those described in: Maliszewski, *J Immunol* 144:3028-3033, 1990; and Mond and Brunswick In: CURRENT PROTOCOLS IN IMMUNOLOGY. Coligan *et al.*, (eds.) Vol 1 pp. 3.8.1-3.8.16, John Wiley and Sons, Toronto 1994.

Mixed lymphocyte reaction (MLR) assays (which will identify, among others, proteins that
30 generate predominantly Th1 and CTL responses) include, without limitation, those described In: CURRENT PROTOCOLS IN IMMUNOLOGY. Coligan *et al.*, eds. Greene Publishing Associates and Wiley-Interscience (Chapter 3, Chapter 7); Takai *et al.*, *J Immunol* 137:3494-3500, 1986; Takai *et al.*, *J Immunol* 140:508-512, 1988; Bertagnolli *et al.*, *J Immunol* 149:3778-3783, 1992.

Dendritic cell-dependent assays (which will identify, among others, proteins expressed by
35 dendritic cells that activate naive T-cells) include, without limitation, those described in: Guery *et al.*,

J Immunol 134:536-544, 1995; Inaba *et al.*, *J Exp Med* 173:549-559, 1991; Macatonia *et al.*, *J Immunol* 154:5071-5079, 1995; Porgador *et al.*, *J Exp Med* 182:255-260, 1995; Nair *et al.*, *J Virol* 67:4062-4069, 1993; Huang *et al.*, *Science* 264:961-965, 1994; Macatonia *et al.*, *J Exp Med* 169:1255-1264, 1989; Bhardwaj *et al.*, *J Clin Investig* 94:797-807, 1994; and Inaba *et al.*, *J Exp Med* 172:631-640, 1990.

Assays for lymphocyte survival/apoptosis (which will identify, among others, proteins that prevent apoptosis after superantigen induction and proteins that regulate lymphocyte homeostasis) include, without limitation, those described in: Darzynkiewicz *et al.*, *Cytometry* 13:795-808, 1992; Gorczyca *et al.*, *Leukemia* 7:659-670, 1993; Gorczyca *et al.*, *Cancer Res* 53:1945-1951, 1993; Itoh *et al.*, *Cell* 66:233-243, 1991; Zacharchuk, *J Immunol* 145:4037-4045, 1990; Zamai *et al.*, *Cytometry* 14:891-897, 1993; Gorczyca *et al.*, *Internat J Oncol* 1:639-648, 1992.

Assays for proteins that influence early steps of T-cell commitment and development include, without limitation, those described in: Antica *et al.*, *Blood* 84:111-117, 1994; Fine *et al.*, *Cell Immunol* 155: 111-122, 1994; Galy *et al.*, *Blood* 85:2770-2778, 1995; Toki *et al.*, *Proc Nat Acad Sci USA* 88:7548-7551, 1991.

Hematopoiesis Regulating Activity

A SECX protein of the present invention may be useful in regulation of hematopoiesis and, consequently, in the treatment of myeloid or lymphoid cell deficiencies. Even marginal biological activity in support of colony forming cells or of factor-dependent cell lines indicates involvement in regulating hematopoiesis, *e.g.* in supporting the growth and proliferation of erythroid progenitor cells alone or in combination with other cytokines, thereby indicating utility, for example, in treating various anemias or for use in conjunction with irradiation/chemotherapy to stimulate the production of erythroid precursors and/or erythroid cells; in supporting the growth and proliferation of myeloid cells such as granulocytes and monocytes/macrophages (*i.e.*, traditional CSF activity) useful, for example, in conjunction with chemotherapy to prevent or treat consequent myelo-suppression; in supporting the growth and proliferation of megakaryocytes and consequently of platelets thereby allowing prevention or treatment of various platelet disorders such as thrombocytopenia, and generally for use in place of or complimentary to platelet transfusions; and/or in supporting the growth and proliferation of hematopoietic stem cells which are capable of maturing to any and all of the above-mentioned hematopoietic cells and therefore find therapeutic utility in various stem cell disorders (such as those usually treated with transplantation, including, without limitation, aplastic anemia and paroxysmal nocturnal hemoglobinuria), as well as in repopulating the stem cell compartment post irradiation/chemotherapy, either *in-vivo* or *ex-vivo* (*i.e.*, in conjunction with bone marrow transplantation or with peripheral progenitor cell transplantation (homologous or heterologous)) as normal cells or genetically manipulated for gene therapy.

The activity of a protein of the invention may, among other means, be measured by the following methods:

Suitable assays for proliferation and differentiation of various hematopoietic lines are cited above.

5 Assays for embryonic stem cell differentiation (which will identify, among others, proteins that influence embryonic differentiation hematopoiesis) include, without limitation, those described in: Johansson *et al.* *Cellular Biology* 15:141-151, 1995; Keller *et al.*, *Mol. Cell. Biol.* 13:473-486, 1993; McClanahan *et al.*, *Blood* 81:2903-2915, 1993.

10 Assays for stem cell survival and differentiation (which will identify, among others, proteins that regulate lympho-hematopoiesis) include, without limitation, those described in: Methylcellulose colony forming assays, Freshney, In: CULTURE OF HEMATOPOIETIC CELLS. Freshney, *et al.* (eds.) Vol pp. 265-268, Wiley-Liss, Inc., New York, N.Y 1994; Hirayama *et al.*, *Proc Natl Acad Sci USA* 89:5907-5911, 1992; McNiece and Briddeli, In: CULTURE OF HEMATOPOIETIC CELLS. Freshney, *et al.* (eds.) Vol pp. 23-39, Wiley-Liss, Inc., New York, N.Y. 1994; Neben *et al.*, *Exp Hematol* 22:353-359, 1994; Ploemacher, In: CULTURE OF HEMATOPOIETIC CELLS. Freshney, *et al.* eds. Vol pp. 1-21, Wiley-Liss, Inc., New York, N.Y. 1994; Spooncer *et al.*, In: CULTURE OF HEMATOPOIETIC CELLS. Freshney, *et al.*, (eds.) Vol pp. 163-179, Wiley-Liss, Inc., New York, N.Y. 1994; Sutherland, In: CULTURE OF HEMATOPOIETIC CELLS. Freshney, *et al.*, (eds.) Vol pp. 139-162, Wiley-Liss, Inc., New York, N.Y. 1994.

20 **Tissue Growth Activity**

A SECX protein of the present invention also may have utility in compositions used for bone, cartilage, tendon, ligament and/or nerve tissue growth or regeneration, as well as for wound healing and tissue repair and replacement, and in the treatment of burns, incisions and ulcers.

25 A protein of the present invention, which induces cartilage and/or bone growth in circumstances where bone is not normally formed, has application in the healing of bone fractures and cartilage damage or defects in humans and other animals. Such a preparation employing a protein of the invention may have prophylactic use in closed as well as open fracture reduction and also in the improved fixation of artificial joints. De novo bone formation induced by an osteogenic agent contributes to the repair of congenital, trauma induced, or oncologic resection induced craniofacial defects, and also is useful in cosmetic plastic surgery.

30 A protein of this invention may also be used in the treatment of periodontal disease, and in other tooth repair processes. Such agents may provide an environment to attract bone-forming cells, stimulate growth of bone-forming cells or induce differentiation of progenitors of bone-forming cells. A protein of the invention may also be useful in the treatment of osteoporosis or osteoarthritis, such as

through stimulation of bone and/or cartilage repair or by blocking inflammation or processes of tissue destruction (collagenase activity, osteoclast activity, etc.) mediated by inflammatory processes.

Another category of tissue regeneration activity that may be attributable to the protein of the present invention is tendon/ligament formation. A protein of the present invention, which induces tendon/ligament-like tissue or other tissue formation in circumstances where such tissue is not normally formed, has application in the healing of tendon or ligament tears, deformities and other tendon or ligament defects in humans and other animals. Such a preparation employing a tendon/ligament-like tissue inducing protein may have prophylactic use in preventing damage to tendon or ligament tissue, as well as use in the improved fixation of tendon or ligament to bone or other tissues, and in repairing defects to tendon or ligament tissue. De novo tendon/ligament-like tissue formation induced by a composition of the present invention contributes to the repair of congenital, trauma induced, or other tendon or ligament defects of other origin, and is also useful in cosmetic plastic surgery for attachment or repair of tendons or ligaments. The compositions of the present invention may provide an environment to attract tendon- or ligament-forming cells, stimulate growth of tendon- or ligament-forming cells, induce differentiation of progenitors of tendon- or ligament-forming cells, or induce growth of tendon/ligament cells or progenitors *ex vivo* for return *in vivo* to effect tissue repair. The compositions of the invention may also be useful in the treatment of tendonitis, carpal tunnel syndrome and other tendon or ligament defects. The compositions may also include an appropriate matrix and/or sequestering agent as a carrier as is well known in the art.

The protein of the present invention may also be useful for proliferation of neural cells and for regeneration of nerve and brain tissue, *i.e.* for the treatment of central and peripheral nervous system diseases and neuropathies, as well as mechanical and traumatic disorders, which involve degeneration, death or trauma to neural cells or nerve tissue. More specifically, a protein may be used in the treatment of diseases of the peripheral nervous system, such as peripheral nerve injuries, peripheral neuropathy and localized neuropathies, and central nervous system diseases, such as Alzheimer's, Parkinson's disease, Huntington's disease, amyotrophic lateral sclerosis, and Shy-Drager syndrome. Further conditions which may be treated in accordance with the present invention include mechanical and traumatic disorders, such as spinal cord disorders, head trauma and cerebrovascular diseases such as stroke. Peripheral neuropathies resulting from chemotherapy or other medical therapies may also be treatable using a protein of the invention.

Proteins of the invention may also be useful to promote better or faster closure of non-healing wounds, including without limitation pressure ulcers, ulcers associated with vascular insufficiency, surgical and traumatic wounds, and the like.

It is expected that a protein of the present invention may also exhibit activity for generation or regeneration of other tissues, such as organs (including, for example, pancreas, liver, intestine, kidney, skin, endothelium), muscle (smooth, skeletal or cardiac) and vascular (including vascular

endothelium) tissue, or for promoting the growth of cells comprising such tissues. Part of the desired effects may be by inhibition or modulation of fibrotic scarring to allow normal tissue to regenerate. A protein of the invention may also exhibit angiogenic activity.

5 A protein of the present invention may also be useful for gut protection or regeneration and treatment of lung or liver fibrosis, reperfusion injury in various tissues, and conditions resulting from systemic cytokine damage.

A protein of the present invention may also be useful for promoting or inhibiting differentiation of tissues described above from precursor tissues or cells; or for inhibiting the growth of tissues described above.

10 The activity of a protein of the invention may, among other means, be measured by the following methods:

Assays for tissue generation activity include, without limitation, those described in: International Patent Publication No. WO95/16035 (bone, cartilage, tendon); International Patent Publication No. WO95/05846 (nerve, neuronal); International Patent Publication No. WO91/07491
15 (skin, endothelium).

Assays for wound healing activity include, without limitation, those described in: Winter, EPIDERMAL WOUND HEALING, pp. 71-112 (Maibach and Rovee, eds.), Year Book Medical Publishers, Inc., Chicago, as modified by Eaglstein and Menz, *J. Invest. Dermatol* 71:382-84 (1978).

Activin/Inhibin Activity

20 A SECX protein of the present invention may also exhibit activin- or inhibin-related activities. Inhibins are characterized by their ability to inhibit the release of follicle stimulating hormone (FSH), while activins and are characterized by their ability to stimulate the release of follicle stimulating hormone (FSH). Thus, a protein of the present invention, alone or in heterodimers with a member of the inhibin a family, may be useful as a contraceptive based on the ability of inhibins to decrease
25 fertility in female mammals and decrease spermatogenesis in male mammals. Administration of sufficient amounts of other inhibins can induce infertility in these mammals. Alternatively, the protein of the invention, as a homodimer or as a heterodimer with other protein subunits of the inhibin-b group, may be useful as a fertility inducing therapeutic, based upon the ability of activin molecules in stimulating FSH release from cells of the anterior pituitary. See, for example, U.S. Pat. No. 4,798,885.
30 A protein of the invention may also be useful for advancement of the onset of fertility in sexually immature mammals, so as to increase the lifetime reproductive performance of domestic animals such as cows, sheep and pigs.

The activity of a protein of the invention may, among other means, be measured by the following methods:

Assays for activin/inhibin activity include, without limitation, those described in: Vale *et al.*, *Endocrinology* 91:562-572, 1972; Ling *et al.*, *Nature* 321:779-782, 1986; Vale *et al.*, *Nature* 321:776-779, 1986; Mason *et al.*, *Nature* 318:659-663, 1985; Forage *et al.*, *Proc Natl Acad Sci USA* 83:3091-3095, 1986.

5 **Chemotactic/Chemokinetic Activity**

A protein of the present invention may have chemotactic or chemokinetic activity (e.g., act as a chemokine) for mammalian cells, including, for example, monocytes, fibroblasts, neutrophils, T-cells, mast cells, eosinophils, epithelial and/or endothelial cells. Chemotactic and chemokinetic proteins can be used to mobilize or attract a desired cell population to a desired site of action.

10 Chemotactic or chemokinetic proteins provide particular advantages in treatment of wounds and other trauma to tissues, as well as in treatment of localized infections. For example, attraction of lymphocytes, monocytes or neutrophils to tumors or sites of infection may result in improved immune responses against the tumor or infecting agent.

15 A protein or peptide has chemotactic activity for a particular cell population if it can stimulate, directly or indirectly, the directed orientation or movement of such cell population. Preferably, the protein or peptide has the ability to directly stimulate directed movement of cells. Whether a particular protein has chemotactic activity for a population of cells can be readily determined by employing such protein or peptide in any known assay for cell chemotaxis.

20 The activity of a protein of the invention may, among other means, be measured by following methods. Assays for chemotactic activity (which will identify proteins that induce or prevent chemotaxis) consist of assays that measure the ability of a protein to induce the migration of cells across a membrane as well as the ability of a protein to induce the adhesion of one cell population to another cell population. Suitable assays for movement and adhesion include, without limitation, those described in: CURRENT PROTOCOLS IN IMMUNOLOGY, Coligan *et al.*, eds. (Chapter 6.12, 25 MEASUREMENT OF ALPHA AND BETA CHEMOKINES 6.12.1-6.12.28); Taub *et al.* *J Clin Invest* 95:1370-1376, 1995; Lind *et al.* *APMIS* 103:140-146, 1995; Muller *et al.*, *Eur J Immunol* 25: 1744-1748; Gruber *et al.* *J Immunol* 152:5860-5867, 1994; Johnston *et al.*, *J Immunol* 153: 1762-1768, 1994.

20 **Hemostatic and Thrombolytic Activity**

30 A protein of the invention may also exhibit hemostatic or thrombolytic activity. As a result, such a protein is expected to be useful in treatment of various coagulation disorders (including hereditary disorders, such as hemophilias) or to enhance coagulation and other hemostatic events in treating wounds resulting from trauma, surgery or other causes. A protein of the invention may also be useful for dissolving or inhibiting formation of thromboses and for treatment and prevention of

conditions resulting therefrom (such as, for example, infarction of cardiac and central nervous system vessels (e.g., stroke).

The activity of a protein of the invention may, among other means, be measured by the following methods:

- 5 Assay for hemostatic and thrombolytic activity include, without limitation, those described in: Linet *et al.*, *J. Clin. Pharmacol.* 26:131-140, 1986; Burdick *et al.*, *Thrombosis Res.* 45:413-419, 1987; Humphrey *et al.*, *Fibrinolysis* 5:71-79 (1991); Schaub, *Prostaglandins* 35:467-474, 1988.

Receptor/Ligand Activity

A protein of the present invention may also demonstrate activity as receptors, receptor ligands or inhibitors or agonists of receptor/ligand interactions. Examples of such receptors and ligands include, without limitation, cytokine receptors and their ligands, receptor kinases and their ligands, receptor phosphatases and their ligands, receptors involved in cell—cell interactions and their ligands (including without limitation, cellular adhesion molecules (such as selectins, integrins and their ligands) and receptor/ligand pairs involved in antigen presentation, antigen recognition and development of cellular and humoral immune responses). Receptors and ligands are also useful for screening of potential peptide or small molecule inhibitors of the relevant receptor/ligand interaction. A protein of the present invention (including, without limitation, fragments of receptors and ligands) may themselves be useful as inhibitors of receptor/ligand interactions.

The activity of a protein of the invention may, among other means, be measured by the following methods:

- Suitable assays for receptor-ligand activity include without limitation those described in: CURRENT PROTOCOLS IN IMMUNOLOGY, Ed by Coligan, *et al.*, Greene Publishing Associates and Wiley-Interscience (Chapter 7.28, Measurement of Cellular Adhesion under static conditions 7.28.1-7.28.22), Takai *et al.*, *Proc Natl Acad Sci USA* 84:6864-6868, 1987; Bierer *et al.*, *J. Exp. Med.* 168:1145-1156, 1988; Rosenstein *et al.*, *J. Exp. Med.* 169:149-160 1989; Stoltenborg *et al.*, *J Immunol Methods* 175:59-68, 1994; Stitt *et al.*, *Cell* 80:661-670, 1995.

Anti-Inflammatory Activity

Proteins of the present invention may also exhibit anti-inflammatory activity. The anti-inflammatory activity may be achieved by providing a stimulus to cells involved in the inflammatory response, by inhibiting or promoting cell—cell interactions (such as, for example, cell adhesion), by inhibiting or promoting chemotaxis of cells involved in the inflammatory process, inhibiting or promoting cell extravasation, or by stimulating or suppressing production of other factors which more directly inhibit or promote an inflammatory response. Proteins exhibiting such activities can be used to treat inflammatory conditions including chronic or acute conditions), including without limitation inflammation associated with infection (such as septic shock, sepsis or systemic

inflammatory response syndrome (SIRS)), ischemia-reperfusion injury, endotoxin lethality, arthritis, complement-mediated hyperacute rejection, nephritis, cytokine or chemokine-induced lung injury, inflammatory bowel disease, Crohn's disease or resulting from over production of cytokines such as TNF or IL-1. Proteins of the invention may also be useful to treat anaphylaxis and hypersensitivity to an antigenic substance or material.

5 **Tumor Inhibition Activity**

In addition to the activities described above for immunological treatment or prevention of tumors, a protein of the invention may exhibit other anti-tumor activities. A protein may inhibit tumor growth directly or indirectly (such as, for example, via ADCC). A protein may exhibit its tumor inhibitory activity by acting on tumor tissue or tumor precursor tissue, by inhibiting formation of tissues necessary to support tumor growth (such as, for example, by inhibiting angiogenesis), by causing production of other factors, agents or cell types which inhibit tumor growth, or by suppressing, eliminating or inhibiting factors, agents or cell types which promote tumor growth.

10 **Other Activities**

A protein of the invention may also exhibit one or more of the following additional activities or effects: inhibiting the growth, infection or function of, or killing, infectious agents, including, without limitation, bacteria, viruses, fungi and other parasites; effecting (suppressing or enhancing) bodily characteristics, including, without limitation, height, weight, hair color, eye color, skin, fat to lean ratio or other tissue pigmentation, or organ or body part size or shape (such as, for example, breast augmentation or diminution, change in bone form or shape); effecting biorhythms or circadian cycles or rhythms; effecting the fertility of male or female subjects; effecting the metabolism, catabolism, anabolism, processing, utilization, storage or elimination of dietary fat, lipid, protein, carbohydrate, vitamins, minerals, cofactors or other nutritional factors or component(s); effecting behavioral characteristics, including, without limitation, appetite, libido, stress, cognition (including cognitive disorders), depression (including depressive disorders) and violent behaviors; providing analgesic effects or other pain reducing effects; promoting differentiation and growth of embryonic stem cells in lineages other than hematopoietic lineages; hormonal or endocrine activity; in the case of enzymes, correcting deficiencies of the enzyme and treating deficiency-related diseases; treatment of hyperproliferative disorders (such as, for example, psoriasis); immunoglobulin-like activity (such as, for example, the ability to bind antigens or complement); and the ability to act as an antigen in a vaccine composition to raise an immune response against such protein or another material or entity which is cross-reactive with such protein.

30 **Predictive Medicine**

The present invention also pertains to the field of predictive medicine in which diagnostic assays, prognostic assays, pharmacogenomics, and monitoring clinical trials are used for prognostic

(predictive) purposes to thereby treat an individual prophylactically. Accordingly, one aspect of the present invention relates to diagnostic assays for determining SECX protein and/or nucleic acid expression as well as SECX activity, in the context of a biological sample (e.g., blood, serum, cells, tissue) to thereby determine whether an individual is afflicted with a disease or disorder, or is at risk of developing a disorder, associated with aberrant SECX expression or activity. The invention also provides for prognostic (or predictive) assays for determining whether an individual is at risk of developing a disorder associated with SECX protein, nucleic acid expression or activity. For example, mutations in a SECX gene can be assayed in a biological sample. Such assays can be used for prognostic or predictive purpose to thereby prophylactically treat an individual prior to the onset of a disorder characterized by or associated with SECX protein, nucleic acid expression or activity.

Another aspect of the invention provides methods for determining SECX protein, nucleic acid expression or SECX activity in an individual to thereby select appropriate therapeutic or prophylactic agents for that individual (referred to herein as "pharmacogenomics"). Pharmacogenomics allows for the selection of agents (e.g., drugs) for therapeutic or prophylactic treatment of an individual based on the genotype of the individual (e.g., the genotype of the individual examined to determine the ability of the individual to respond to a particular agent.)

Yet another aspect of the invention pertains to monitoring the influence of agents (e.g., drugs, compounds) on the expression or activity of SECX in clinical trials.

These and other agents are described in further detail in the following sections.

20 **Diagnostic Assays**

An exemplary method for detecting the presence or absence of SECX in a biological sample involves obtaining a biological sample from a test subject and contacting the biological sample with a compound or an agent capable of detecting SECX protein or nucleic acid (e.g., mRNA, genomic DNA) that encodes SECX protein such that the presence of SECX is detected in the biological sample. An agent for detecting SECX mRNA or genomic DNA is a labeled nucleic acid probe capable of hybridizing to SECX mRNA or genomic DNA. The nucleic acid probe can be, for example, a full-length SECX nucleic acid, or a portion thereof, such as an oligonucleotide of at least 15, 30, 50, 100, 250 or 500 nucleotides in length and sufficient to specifically hybridize under stringent conditions to SECX mRNA or genomic DNA. Other suitable probes for use in the diagnostic assays of the invention are described herein.

An agent for detecting SECX protein is an antibody capable of binding to SECX protein, preferably an antibody with a detectable label. Antibodies can be polyclonal, or more preferably, monoclonal. An intact antibody, or a fragment thereof (e.g., Fab or F(ab')₂) can be used. The term "labeled", with regard to the probe or antibody, is intended to encompass direct labeling of the probe or antibody by coupling (*i.e.*, physically linking) a detectable substance to the probe or antibody, as

well as indirect labeling of the probe or antibody by reactivity with another reagent that is directly labeled. Examples of indirect labeling include detection of a primary antibody using a fluorescently labeled secondary antibody and end-labeling of a DNA probe with biotin such that it can be detected with fluorescently labeled streptavidin. The term "biological sample" is intended to include tissues, 5 cells and biological fluids isolated from a subject, as well as tissues, cells and fluids present within a subject. That is, the detection method of the invention can be used to detect SECX mRNA, protein, or genomic DNA in a biological sample *in vitro* as well as *in vivo*. For example, *in vitro* techniques for detection of SECX mRNA include Northern hybridizations and *in situ* hybridizations. *In vitro* techniques for detection of SECX protein include enzyme linked immunosorbent assays (ELISAs), 10 Western blots, immunoprecipitations and immunofluorescence. *In vitro* techniques for detection of SECX genomic DNA include Southern hybridizations. Furthermore, *in vivo* techniques for detection of SECX protein include introducing into a subject a labeled anti-SECX antibody. For example, the antibody can be labeled with a radioactive marker whose presence and location in a subject can be detected by standard imaging techniques.

15 In one embodiment, the biological sample contains protein molecules from the test subject. Alternatively, the biological sample can contain mRNA molecules from the test subject or genomic DNA molecules from the test subject. A preferred biological sample is a peripheral blood leukocyte sample isolated by conventional means from a subject.

20 In another embodiment, the methods further involve obtaining a control biological sample from a control subject, contacting the control sample with a compound or agent capable of detecting SECX protein, mRNA, or genomic DNA, such that the presence of SECX protein, mRNA or genomic DNA is detected in the biological sample, and comparing the presence of SECX protein, mRNA or genomic DNA in the control sample with the presence of SECX protein, mRNA or genomic DNA in the test sample.

25 The invention also encompasses kits for detecting the presence of SECX in a biological sample. For example, the kit can comprise: a labeled compound or agent capable of detecting SECX protein or mRNA in a biological sample; means for determining the amount of SECX in the sample; and means for comparing the amount of SECX in the sample with a standard. The compound or agent can be packaged in a suitable container. The kit can further comprise instructions for using the kit to 30 detect SECX protein or nucleic acid.

Prognostic Assays

The diagnostic methods described herein can furthermore be utilized to identify subjects having or at risk of developing a disease or disorder associated with aberrant SECX expression or activity. For example, the assays described herein, such as the preceding diagnostic assays or the 35 following assays, can be utilized to identify a subject having or at risk of developing a disorder

associated with SECX protein, nucleic acid expression or activity such as cancer or fibrotic disorders, or a SECX-specific disease as described in the individual sections 1-14, above. Alternatively, the prognostic assays can be utilized to identify a subject having or at risk for developing a disease or disorder. Thus, the present invention provides a method for identifying a disease or disorder associated
5 with aberrant SECX expression or activity in which a test sample is obtained from a subject and SECX protein or nucleic acid (*e.g.*, mRNA, genomic DNA) is detected, wherein the presence of SECX protein or nucleic acid is diagnostic for a subject having or at risk of developing a disease or disorder associated with aberrant SECX expression or activity. As used herein, a "test sample" refers to a biological sample obtained from a subject of interest. For example, a test sample can be a biological
10 fluid (*e.g.*, serum), cell sample, or tissue.

Furthermore, the prognostic assays described herein can be used to determine whether a subject can be administered an agent (*e.g.*, an agonist, antagonist, peptidomimetic, protein, peptide, nucleic acid, small molecule, or other drug candidate) to treat a disease or disorder associated with aberrant SECX expression or activity. For example, such methods can be used to determine whether a
15 subject can be effectively treated with an agent for a disorder, such as cancer or preclampsia or a SECX-specific disease as described in the individual sections 1-14, above. Thus, the present invention provides methods for determining whether a subject can be effectively treated with an agent for a disorder associated with aberrant SECX expression or activity in which a test sample is obtained and SECX protein or nucleic acid is detected (*e.g.*, wherein the presence of SECX protein or nucleic acid
20 is diagnostic for a subject that can be administered the agent to treat a disorder associated with aberrant SECX expression or activity.)

The methods of the invention can also be used to detect genetic lesions in a SECX gene, thereby determining if a subject with the lesioned gene is at risk for a disorder characterized by aberrant cell proliferation and/or differentiation. In various embodiments, the methods include
25 detecting, in a sample of cells from the subject, the presence or absence of a genetic lesion characterized by at least one of an alteration affecting the integrity of a gene encoding a SECX-protein, or the mis-expression of the SECX gene. For example, such genetic lesions can be detected by ascertaining the existence of at least one of (1) a deletion of one or more nucleotides from a SECX gene; (2) an addition of one or more nucleotides to a SECX gene; (3) a substitution of one or
30 more nucleotides of a SECX gene, (4) a chromosomal rearrangement of a SECX gene; (5) an alteration in the level of a messenger RNA transcript of a SECX gene, (6) aberrant modification of a SECX gene, such as of the methylation pattern of the genomic DNA, (7) the presence of a non-wild type splicing pattern of a messenger RNA transcript of a SECX gene, (8) a non-wild type level of a SECX-protein, (9) allelic loss of a SECX gene, and (10) inappropriate post-translational modification
35 of a SECX-protein. As described herein, there are a large number of assay techniques known in the art which can be used for detecting lesions in a SECX gene. A preferred biological sample is a peripheral

blood leukocyte sample isolated by conventional means from a subject. However, any biological sample containing nucleated cells may be used, including, for example, buccal mucosal cells.

In certain embodiments, detection of the lesion involves the use of a probe/primer in a polymerase chain reaction (PCR) (see, e.g., U.S. Pat. Nos. 4,683,195 and 4,683,202), such as anchor 5 PCR or RACE PCR, or, alternatively, in a ligation chain reaction (LCR) (see, e.g., Landegran *et al.* (1988) *Science* 241:1077-1080; and Nakazawa *et al.* (1994) *PNAS* 91:360-364), the latter of which can be particularly useful for detecting point mutations in the SECX-gene (see Abravaya *et al.* (1995) *Nucl Acids Res* 23:675-682). This method can include the steps of collecting a sample of cells from a patient, isolating nucleic acid (e.g., genomic, mRNA or both) from the cells of the sample, contacting 10 the nucleic acid sample with one or more primers that specifically hybridize to a SECX gene under conditions such that hybridization and amplification of the SECX gene (if present) occurs, and detecting the presence or absence of an amplification product, or detecting the size of the amplification product and comparing the length to a control sample. It is anticipated that PCR and/or LCR may be desirable to use as a preliminary amplification step in conjunction with any of the 15 techniques used for detecting mutations described herein.

Alternative amplification methods include: self sustained sequence replication (Guatelli *et al.*, 1990, *Proc Natl Acad Sci USA* 87:1874-1878), transcriptional amplification system (Kwoh, *et al.*, 1989, *Proc Natl Acad Sci USA* 86:1173-1177), Q-Beta Replicase (Lizardi *et al.*, 1988, *BioTechnology* 6:1197), or any other nucleic acid amplification method, followed by the detection of the amplified 20 molecules using techniques well known to those of skill in the art. These detection schemes are especially useful for the detection of nucleic acid molecules if such molecules are present in very low numbers.

In an alternative embodiment, mutations in a SECX gene from a sample cell can be identified by alterations in restriction enzyme cleavage patterns. For example, sample and control DNA is 25 isolated, amplified (optionally), digested with one or more restriction endonucleases, and fragment length sizes are determined by gel electrophoresis and compared. Differences in fragment length sizes between sample and control DNA indicates mutations in the sample DNA. Moreover, the use of sequence specific ribozymes (see, for example, U.S. Pat. No. 5,493,531) can be used to score for the presence of specific mutations by development or loss of a ribozyme cleavage site.

In other embodiments, genetic mutations in SECX can be identified by hybridizing a sample 30 and control nucleic acids, e.g., DNA or RNA, to high density arrays containing hundreds or thousands of oligonucleotides probes (Cronin *et al.* (1996) *Human Mutation* 7: 244-255; Kozal *et al.* (1996) *Nature Medicine* 2: 753-759). For example, genetic mutations in SECX can be identified in two-dimensional arrays containing light-generated DNA probes as described in Cronin *et al.* above. 35 Briefly, a first hybridization array of probes can be used to scan through long stretches of DNA in a sample and control to identify base changes between the sequences by making linear arrays of

sequential overlapping probes. This step allows the identification of point mutations. This step is followed by a second hybridization array that allows the characterization of specific mutations by using smaller, specialized probe arrays complementary to all variants or mutations detected. Each mutation array is composed of parallel probe sets, one complementary to the wild-type gene and the other complementary to the mutant gene.

In yet another embodiment, any of a variety of sequencing reactions known in the art can be used to directly sequence the SECX gene and detect mutations by comparing the sequence of the sample SECX with the corresponding wild-type (control) sequence. Examples of sequencing reactions include those based on techniques developed by Maxim and Gilbert (1977) *PNAS* 74:560 or Sanger (1977) *PNAS* 74:5463. It is also contemplated that any of a variety of automated sequencing procedures can be utilized when performing the diagnostic assays (Naeve *et al.*, (1995) *BioTechniques* 19:448), including sequencing by mass spectrometry (see, e.g., PCT International Publ. No. WO 94/16101; Cohen *et al.* (1996) *Adv Chromatogr* 36:127-162; and Griffin *et al.* (1993) *Appl Biochem Biotechnol* 38:147-159).

Other methods for detecting mutations in the SECX gene include methods in which protection from cleavage agents is used to detect mismatched bases in RNA/RNA or RNA/DNA heteroduplexes (Myers *et al.* (1985) *Science* 230:1242). In general, the art technique of "mismatch cleavage" starts by providing heteroduplexes of formed by hybridizing (labeled) RNA or DNA containing the wild-type SECX sequence with potentially mutant RNA or DNA obtained from a tissue sample. The double-stranded duplexes are treated with an agent that cleaves single-stranded regions of the duplex such as which will exist due to basepair mismatches between the control and sample strands. For instance, RNA/DNA duplexes can be treated with RNase and DNA/DNA hybrids treated with S1 nuclease to enzymatically digesting the mismatched regions. In other embodiments, either DNA/DNA or RNA/DNA duplexes can be treated with hydroxylamine or osmium tetroxide and with piperidine in order to digest mismatched regions. After digestion of the mismatched regions, the resulting material is then separated by size on denaturing polyacrylamide gels to determine the site of mutation. See, for example, Cotton *et al* (1988) *Proc Natl Acad Sci USA* 85:4397; Saleeba *et al* (1992) *Methods Enzymol* 217:286-295. In an embodiment, the control DNA or RNA can be labeled for detection.

In still another embodiment, the mismatch cleavage reaction employs one or more proteins that recognize mismatched base pairs in double-stranded DNA (so called "DNA mismatch repair" enzymes) in defined systems for detecting and mapping point mutations in SECX cDNAs obtained from samples of cells. For example, the mutY enzyme of *E. coli* cleaves A at G/A mismatches and the thymidine DNA glycosylase from HeLa cells cleaves T at G/T mismatches (Hsu *et al.* (1994) *Carcinogenesis* 15:1657-1662). According to an exemplary embodiment, a probe based on a SECX sequence, e.g., a wild-type SECX sequence, is hybridized to a cDNA or other DNA product from a test

cell(s). The duplex is treated with a DNA mismatch repair enzyme, and the cleavage products, if any, can be detected from electrophoresis protocols or the like. See, for example, U.S. Pat. No. 5,459,039.

In other embodiments, alterations in electrophoretic mobility will be used to identify mutations in SECX genes. For example, single strand conformation polymorphism (SSCP) may be
5 used to detect differences in electrophoretic mobility between mutant and wild type nucleic acids (Orita *et al.* (1989) *Proc Natl Acad Sci USA*: 86:2766, see also Cotton (1993) *Mutat Res* 285:125-144; Hayashi (1992) *Genet Anal Tech Appl* 9:73-79). Single-stranded DNA fragments of sample and control SECX nucleic acids will be denatured and allowed to renature. The secondary structure of single-stranded nucleic acids varies according to sequence, the resulting alteration in electrophoretic
10 mobility enables the detection of even a single base change. The DNA fragments may be labeled or detected with labeled probes. The sensitivity of the assay may be enhanced by using RNA (rather than DNA), in which the secondary structure is more sensitive to a change in sequence. In one embodiment, the subject method utilizes heteroduplex analysis to separate double stranded heteroduplex molecules on the basis of changes in electrophoretic mobility (Keen *et al.* (1991) *Trends Genet* 7:5).
15

In yet another embodiment the movement of mutant or wild-type fragments in polyacrylamide gels containing a gradient of denaturant is assayed using denaturing gradient gel electrophoresis (DGGE) (Myers *et al.* (1985) *Nature* 313:495). When DGGE is used as the method of analysis, DNA will be modified to insure that it does not completely denature, for example by adding a GC clamp of approximately 40 bp of high-melting GC-rich DNA by PCR. In a further embodiment, a temperature gradient is used in place of a denaturing gradient to identify differences in the mobility of control and sample DNA (Rosenbaum and Reissner (1987) *Biophys Chem* 265:12753).

Examples of other techniques for detecting point mutations include, but are not limited to, selective oligonucleotide hybridization, selective amplification, or selective primer extension. For example, oligonucleotide primers may be prepared in which the known mutation is placed centrally and then hybridized to target DNA under conditions that permit hybridization only if a perfect match is found (Saiki *et al.* (1986) *Nature* 324:163); Saiki *et al.* (1989) *Proc Natl Acad. Sci USA* 86:6230). Such allele specific oligonucleotides are hybridized to PCR amplified target DNA or a number of different mutations when the oligonucleotides are attached to the hybridizing membrane and
30 hybridized with labeled target DNA.

Alternatively, allele specific amplification technology that depends on selective PCR amplification may be used in conjunction with the instant invention. Oligonucleotides used as primers for specific amplification may carry the mutation of interest in the center of the molecule (so that amplification depends on differential hybridization) (Gibbs *et al.* (1989) *Nucleic Acids Res* 17:2437-2448) or at the extreme 3' end of one primer where, under appropriate conditions, mismatch can prevent, or reduce polymerase extension (Prossner (1993) *Tibtech* 11:238). In addition it may be
35

desirable to introduce a novel restriction site in the region of the mutation to create cleavage-based detection (Gasparini *et al* (1992) *Mol Cell Probes* 6:1). It is anticipated that in certain embodiments amplification may also be performed using Taq ligase for amplification (Barany (1991) *Proc Natl Acad Sci USA* 88:189). In such cases, ligation will occur only if there is a perfect match at the 3' end of 5 the 5' sequence, making it possible to detect the presence of a known mutation at a specific site by looking for the presence or absence of amplification.

The methods described herein may be performed, for example, by utilizing pre-packaged diagnostic kits comprising at least one probe nucleic acid or antibody reagent described herein, which may be conveniently used, e.g., in clinical settings to diagnose patients exhibiting symptoms or family 10 history of a disease or illness involving a SECX gene.

Furthermore, any cell type or tissue, preferably peripheral blood leukocytes, in which SECX is expressed may be utilized in the prognostic assays described herein. However, any biological sample containing nucleated cells may be used, including, for example, buccal mucosal cells.

Pharmacogenomics

15 Agents, or modulators that have a stimulatory or inhibitory effect on SECX activity (e.g., SECX gene expression), as identified by a screening assay described herein can be administered to individuals to treat (prophylactically or therapeutically) disorders (e.g., cancer or gestational disorders or a SECX-specific disease as described in the individual sections 1-14, above) associated with aberrant SECX activity. In conjunction with such treatment, the pharmacogenomics (*i.e.*, the study of 20 the relationship between an individual's genotype and that individual's response to a foreign compound or drug) of the individual may be considered. Differences in metabolism of therapeutics can lead to severe toxicity or therapeutic failure by altering the relation between dose and blood concentration of the pharmacologically active drug. Thus, the pharmacogenomics of the individual permits the selection of effective agents (e.g., drugs) for prophylactic or therapeutic treatments based on a 25 consideration of the individual's genotype. Such pharmacogenomics can further be used to determine appropriate dosages and therapeutic regimens. Accordingly, the activity of SECX protein, expression of SECX nucleic acid, or mutation content of SECX genes in an individual can be determined to thereby select appropriate agent(s) for therapeutic or prophylactic treatment of the individual.

30 Pharmacogenomics deals with clinically significant hereditary variations in the response to drugs due to altered drug disposition and abnormal action in affected persons. See e.g., Eichelbaum, *Clin Exp Pharmacol Physiol*, 1996, 23:983-985 and Linder, *Clin Chem*, 1997, 43:254-266. In general, two types of pharmacogenetic conditions can be differentiated. Genetic conditions transmitted as a single factor altering the way drugs act on the body (altered drug action) or genetic conditions transmitted as single factors altering the way the body acts on drugs (altered drug metabolism). These 35 pharmacogenetic conditions can occur either as rare defects or as polymorphisms. For example,

glucose-6-phosphate dehydrogenase (G6PD) deficiency is a common inherited enzymopathy in which the main clinical complication is haemolysis after ingestion of oxidant drugs (anti-malarials, sulfonamides, analgesics, nitrofurans) and consumption of fava beans.

As an illustrative embodiment, the activity of drug metabolizing enzymes is a major determinant of both the intensity and duration of drug action. The discovery of genetic polymorphisms of drug metabolizing enzymes (*e.g.*, N-acetyltransferase 2 (NAT 2) and cytochrome P450 enzymes CYP2D6 and CYP2C19) has provided an explanation as to why some patients do not obtain the expected drug effects or show exaggerated drug response and serious toxicity after taking the standard and safe dose of a drug. These polymorphisms are expressed in two phenotypes in the population, the extensive metabolizer (EM) and poor metabolizer (PM). The prevalence of PM is different among different populations. For example, the gene coding for CYP2D6 is highly polymorphic and several mutations have been identified in PM, which all lead to the absence of functional CYP2D6. Poor metabolizers of CYP2D6 and CYP2C19 quite frequently experience exaggerated drug response and side effects when they receive standard doses. If a metabolite is the active therapeutic moiety, PM show no therapeutic response, as demonstrated for the analgesic effect of codeine mediated by its CYP2D6-formed metabolite morphine. The other extreme are the so called ultra-rapid metabolizers who do not respond to standard doses. Recently, the molecular basis of ultra-rapid metabolism has been identified to be due to CYP2D6 gene amplification.

Thus, the activity of SECX protein, expression of SECX nucleic acid, or mutation content of SECX genes in an individual can be determined to thereby select appropriate agent(s) for therapeutic or prophylactic treatment of the individual. In addition, pharmacogenetic studies can be used to apply genotyping of polymorphic alleles encoding drug-metabolizing enzymes to the identification of an individual's drug responsiveness phenotype. This knowledge, when applied to dosing or drug selection, can avoid adverse reactions or therapeutic failure and thus enhance therapeutic or prophylactic efficiency when treating a subject with a SECX modulator, such as a modulator identified by one of the exemplary screening assays described herein.

Monitoring of Effects During Clinical Trials

Monitoring the influence of agents (*e.g.*, drugs, compounds) on the expression or activity of SECX (*e.g.*, the ability to modulate aberrant cell proliferation and/or differentiation) can be applied not only in basic drug screening, but also in clinical trials. For example, the effectiveness of an agent determined by a screening assay as described herein to increase SECX gene expression, protein levels, or upregulate SECX activity, can be monitored in clinical trials of subjects exhibiting decreased SECX gene expression, protein levels, or downregulated SECX activity. Alternatively, the effectiveness of an agent determined by a screening assay to decrease SECX gene expression, protein levels, or downregulate SECX activity, can be monitored in clinical trials of subjects exhibiting increased SECX

gene expression, protein levels, or upregulated SECX activity. In such clinical trials, the expression or activity of SECX and, preferably, other genes that have been implicated in, for example, a cellular proliferation disorder or a SECX-specific disease as described in the individual sections 1-14, above, can be used as a "read out" or markers of the immune responsiveness of a particular cell.

5 For example, and not by way of limitation, genes, including SECX, that are modulated in cells by treatment with an agent (*e.g.*, compound, drug or small molecule) that modulates SECX activity (*e.g.*, identified in a screening assay as described herein) can be identified. Thus, to study the effect of agents on cellular proliferation disorders, for example, in a clinical trial, cells can be isolated and RNA prepared and analyzed for the levels of expression of SECX and other genes implicated in the
10 disorder. The levels of gene expression (*i.e.*, a gene expression pattern) can be quantified by Northern blot analysis or RT-PCR, as described herein, or alternatively by measuring the amount of protein produced, by one of the methods as described herein, or by measuring the levels of activity of SECX or other genes. In this way, the gene expression pattern can serve as a marker, indicative of the physiological response of the cells to the agent. Accordingly, this response state may be determined
15 before, and at various points during, treatment of the individual with the agent.

In one embodiment, the present invention provides a method for monitoring the effectiveness of treatment of a subject with an agent (*e.g.*, an agonist, antagonist, protein, peptide, peptidomimetic, nucleic acid, small molecule, or other drug candidate identified by the screening assays described herein) comprising the steps of (*i*) obtaining a pre-administration sample from a subject prior to
20 administration of the agent; (*ii*) detecting the level of expression of a SECX protein, mRNA, or genomic DNA in the preadministration sample; (*iii*) obtaining one or more post-administration samples from the subject; (*iv*) detecting the level of expression or activity of the SECX protein, mRNA, or genomic DNA in the post-administration samples; (*v*) comparing the level of expression or activity of the SECX protein, mRNA, or genomic DNA in the pre-administration sample with the
25 SECX protein, mRNA, or genomic DNA in the post administration sample or samples; and (*vi*) altering the administration of the agent to the subject accordingly. For example, increased administration of the agent may be desirable to increase the expression or activity of SECX to higher levels than detected, *i.e.*, to increase the effectiveness of the agent. Alternatively, decreased administration of the agent may be desirable to decrease expression or activity of SECX to lower
30 levels than detected, *i.e.*, to decrease the effectiveness of the agent.

Methods of Treatment

The present invention provides for both prophylactic and therapeutic methods of treating a subject at risk of (or susceptible to) a disorder or having a disorder associated with aberrant SECX expression or activity.

Disorders

- Diseases and disorders that are characterized by increased (relative to a subject not suffering from the disease or disorder) levels or biological activity may be treated with Therapeutics that antagonize (*i.e.*, reduce or inhibit) activity. Therapeutics that antagonize activity may be administered 5 in a therapeutic or prophylactic manner. Therapeutics that may be utilized include, but are not limited to, (i) an aforementioned peptide, or analogs, derivatives, fragments or homologs thereof; (ii) antibodies to an aforementioned peptide; (iii) nucleic acids encoding an aforementioned peptide; (iv) administration of antisense nucleic acid and nucleic acids that are "dysfunctional" (*i.e.*, due to a heterologous insertion within the coding sequences of coding sequences to an aforementioned peptide) 10 that are utilized to "knockout" endogenous function of an aforementioned peptide by homologous recombination (see, *e.g.*, Capecchi, 1989, *Science* 244: 1288-1292); or (v) modulators (*i.e.*, inhibitors, agonists and antagonists, including additional peptide mimetic of the invention or antibodies specific to a peptide of the invention) that alter the interaction between an aforementioned peptide and its binding partner.
- 15 Diseases and disorders that are characterized by decreased (relative to a subject not suffering from the disease or disorder) levels or biological activity may be treated with Therapeutics that increase (*i.e.*, are agonists to) activity. Therapeutics that upregulate activity may be administered in a therapeutic or prophylactic manner. Therapeutics that may be utilized include, but are not limited to, an aforementioned peptide, or analogs, derivatives, fragments or homologs thereof; or an agonist that increases bioavailability.

20 Increased or decreased levels can be readily detected by quantifying peptide and/or RNA, by obtaining a patient tissue sample (*e.g.*, from biopsy tissue) and assaying it *in vitro* for RNA or peptide levels, structure and/or activity of the expressed peptides (or mRNAs of an aforementioned peptide). Methods that are well-known within the art include, but are not limited to, immunoassays (*e.g.*, by 25 Western blot analysis, immunoprecipitation followed by sodium dodecyl sulfate (SDS) polyacrylamide gel electrophoresis, immunocytochemistry, etc.) and/or hybridization assays to detect expression of mRNAs (*e.g.*, Northern assays, dot blots, *in situ* hybridization, etc.).

Prophylactic Methods

- In one aspect, the invention provides a method for preventing, in a subject, a disease or 30 condition associated with an aberrant SECX expression or activity, by administering to the subject an agent that modulates SECX expression or at least one SECX activity. Subjects at risk for a disease that is caused or contributed to by aberrant SECX expression or activity can be identified by, for example, any or a combination of diagnostic or prognostic assays as described herein. Administration of a prophylactic agent can occur prior to the manifestation of symptoms characteristic of the SECX 35 aberrancy, such that a disease or disorder is prevented or, alternatively, delayed in its progression.

Depending on the type of SECX aberrancy, for example, a SECX agonist or SECX antagonist agent can be used for treating the subject. The appropriate agent can be determined based on screening assays described herein. The prophylactic methods of the present invention are further discussed in the following subsections.

5 **Therapeutic Methods**

Another aspect of the invention pertains to methods of modulating SECX expression or activity for therapeutic purposes. The modulatory method of the invention involves contacting a cell with an agent that modulates one or more of the activities of SECX protein activity associated with the cell. An agent that modulates SECX protein activity can be an agent as described herein, such as a nucleic acid or a protein, a naturally-occurring cognate ligand of a SECX protein, a peptide, a SECX peptidomimetic, or other small molecule. In one embodiment, the agent stimulates one or more SECX protein activity. Examples of such stimulatory agents include active SECX protein and a nucleic acid molecule encoding SECX that has been introduced into the cell. In another embodiment, the agent inhibits one or more SECX protein activity. Examples of such inhibitory agents include antisense SECX nucleic acid molecules and anti-SECX antibodies. These modulatory methods can be performed *in vitro* (e.g., by culturing the cell with the agent) or, alternatively, *in vivo* (e.g., by administering the agent to a subject). As such, the present invention provides methods of treating an individual afflicted with a disease or disorder characterized by aberrant expression or activity of a SECX protein or nucleic acid molecule. In one embodiment, the method involves administering an agent (e.g., an agent identified by a screening assay described herein), or combination of agents that modulates (e.g., upregulates or downregulates) SECX expression or activity. In another embodiment, the method involves administering a SECX protein or nucleic acid molecule as therapy to compensate for reduced or aberrant SECX expression or activity.

Stimulation of SECX activity is desirable in situations in which SECX is abnormally downregulated and/or in which increased SECX activity is likely to have a beneficial effect. One example of such a situation is where a subject has a disorder characterized by aberrant cell proliferation and/or differentiation (e.g., cancer). Another example of such a situation is where the subject has a gestational disease (e.g., preclampsia). Other diseases of the invention include the SECX-specific diseases as described in the individual sections 1-14, above.

The present invention is not to be limited in scope by the specific embodiments described herein. Indeed, various modifications of the invention in addition to those described herein will become apparent to those skilled in the art from the foregoing description and accompanying figures. Such modifications are intended to fall within the scope of the appended claims.

This invention is further illustrated by the following examples, which should not be construed as limiting. The contents of all references, patents and published patent applications cited throughout this application are hereby incorporated by reference.

EXAMPLES

5 **Example 1. Radiation Hybrid Mapping Identifies the Chromosomal Location of the Clones of the Invention.**

Radiation hybrid mapping using human chromosome markers was carried out for many of the clones described in the present invention. The procedure used to obtain these results is analogous to methods known in the art, for example, Steen, *et al.* 1999 *Genome Research* 9: AP1-AP8. A panel of 10 93 cell clones containing randomized radiation-induced human chromosomal fragments was screened in 96 well plates using PCR primers designed to identify the given clones in a unique fashion. The results are presented in Table 2, which provides the clone number, the chromosome on which the clone is found, the distance in cR from a marker gene to the sought clone, and the identity of the marker gene.

15 **Table 2. Radiation Hybrid Mapping Results for Clones of the Invention**

Clone No.	Chromosome No.	Distance, cR	Marker Gene
2777610	3	564.40	AFM320WD1
2864933-1 and 2864933-2	5	316.40	WI-9907
2982339	3	355.00	AFM320WD1
3911675	10	391.30	IB3079
4004731-1	12	404.60	WI-5272
4035508	11	230.10	WI-4920
4339264	19	311.50	IB1264

Example 2. Molecular Cloning of 2864933-1

The predicted open reading frame for the 2864933-1 protein codes for a 939 amino acid long Type I transmembrane protein with an overall 95% identity to the mouse semaphorin Via protein. The 20 predicted signal peptide sequence is between residues 1-18, and the predicted transmembrane domain is between residues 645-661. A fragment of the cDNA for the 2864933-1 protein, coding for the extracellular domain of predicted mature protein (i.e., after removal of the signal peptide) from residue 19 to 644, has been cloned from human fetal brain cDNA.

The following oligonucleotide primers were designed to amplify the sought mature form of 25 2864933-1 4 by PCR:

2864933 MatF

GGATCC GGT TTC CCA GAA GAT TCT GAG CCA ATC (SEQ ID NO:33)

2864933 F-TOPO-Reverse

CTC GAG CTG GTC GTG GCC TTT GAG GTA ACT TTC (SEQ ID NO:34)

For cloning purposes, the forward primer includes an in frame BamHI restriction site and the reverse primer contains an in frame XhoI restriction site.

5 PCR reactions were set up using 5 ng human fetal brain cDNA. The reaction mixture contained 1 microM of each of the 2864933 MatF and 2864933 F-TOPO-Reverse primers, 5 micromoles dNTP (Clontech Laboratories, Palo Alto CA) and 1 microliter of 50xAdvantage-HF 2 polymerase (Clontech Laboratories, Palo Alto CA) in 50 microliter volume. The following reaction conditions were used:

- 10 a) 96°C 3 minutes
 - b) 96°C 30 seconds denaturation
 - c) 60°C 30 seconds, primer annealing.
 - d) 72°C 3 minutes extension.
- Repeat steps (b)-(d) 45 times
- 15 e) 72°C 10 minutes final extension

The expected amplified product of approximately 1.9 kbp was detected by agarose gel electrophoresis. The fragment was isolated from the gel and ligated to the vector pCR2.1 (Invitrogen, Carlsbad, CA) using M13 Forward, M13 Reverse primers. The cloned insert was sequenced as PCR amplicons using the following gene-specific primers:

- | | | |
|----|---|----------------|
| 20 | 2864933-Seq-0 CACAAGCCAGGACCGAACAA | (SEQ ID NO:35) |
| | 2864933-Seq-1 TGG AAC TAA TGC CTT CAA C | (SEQ ID NO:36) |
| | 2864933-Seq-2 GAG TCCTGGAGAACAGTGGA | (SEQ ID NO:37) |
| | 2864933-Seq-3 ATGAGGCAGTGCCCTCCATC | (SEQ ID NO:38) |
| | 2864933-Seq-4 CCATATTGTGGATGGATAA | (SEQ ID NO:39) |
| 25 | 2864933-Seq-5 GACACTCAATCCAAAGACC | (SEQ ID NO:40) |
| | 2864933-Seq-6 CCATCACGCAGCAGGGCTA | (SEQ ID NO:41) |

The cloned cDNA (SEQ ID NO:29) was verified to have an open reading frame coding for the predicted mature extracellular domain of 2864933 between residues 19 and 644 (SEQ ID NO:30)(FIG. 15). In FIG. 15, the BamHI and XhoI cloning sites, and the amino acids encoded by them (which are therefore not part of the cloned sequence), are in bold font. The construct is called pCR2.1-2864933.

Example 3. Expression of h2864933 in human embryonic kidney 293 cells.

Oligonucleotide primers pSec-V5-His Forward and pSec-V5-His Reverse were designed to amplify a fragment from the pcDNA3.1-V5His (Invitrogen, Carlsbad, CA) expression vector. The PCR product was digested with XhoI and ApaI and ligated into the XhoI/ApaI digested pSecTag2 B vector harboring an Ig kappa leader sequence (Invitrogen, Carlsbad CA). The correct structure of the resulting vector, pSecV5His, was verified by DNA sequence analysis. The vector pSecV5His was digested with PmeI and NheI, and the PmeI-NheI fragment was ligated into the BamHI/Klenow and NheI treated vector pCEP4 (Invitrogen, Carlsbad, CA). The resulting vector was named pCEP4/Sec.

	pSec-V5-His Forward	
10	CTCGTCCTCGAGGGTAAGCCTATCCCTAAC	(SEQ ID NO:42)
	pSec-V5-His Reverse	
	CTCGTCGGGCCCTGATCAGCGGGTTAAAC	(SEQ ID NO:43),

The 2 kb BamHI-XhoI fragment containing the h2864933 sequence was isolated from pCR2.1-2864933 (Example 2) and subcloned into BamHI-XhoI digested pCEP4/Sec to generate expression vector pCEP4/Sec-2864933. The pCEP4/Sec-2864933 vector was transfected into 293 cells using the LipofectaminePlus reagent following the manufacturer's instructions (Gibco/BRL, Life Technologies, Inc., Rockville, MD). The cell pellet and supernatant were harvested 72 hours after transfection and examined for h2864933 expression by Western blotting (reducing conditions) with an anti-V5 antibody. FIG. 16 shows that h2864933 secreted by 293 cells is detected in two bands carrying the V5 epitope at about 70 kDa and about 100-kDa. The 70kDa band is presumed to represent the unglycosylated protein, and corresponds to the M_r expected for the 626 residue clone with the addition of the V5 epitope. The program PROSITE predicts six N-glycosylation sites in the extracellular h286493 domain. It is thought that the 100 kDa band originates from glycosylated form(s) of the protein.

25 Example 4. Molecular Cloning of 3352358-1

The predicted open reading frame of clone 3352358-1 codes for a 653 amino acid residue Type I transmembrane protein with the transmembrane domain predicted to lie between residues 522 and 551. The cDNA coding for the extracellular segment of the predicted mature protein (i.e., after cleavage of the signal peptide), has been cloned.

30 The secretory signal prediction method, GCG:SPSCAN – Eukaryote, predicts a signal peptidase cleavage site for 3352358-1 between residues 41 and 42. Accordingly, the following oligonucleotide primers were designed to PCR amplify the predicted mature extracellular domain of 3352358 from residue 42 to 486:

3352358CForward

CTCGTCGGATCCA ACTGCCCTCCGTCTGCTCGTGCAG (SEQ ID NO:44), and

3352358CReverse

CTCGTCGTCGACCGTAGAGGTGGTATATGCCGGCTG (SEQ ID NO:45).

5 For cloning purposes, the forward primer includes an in frame BamHI restriction site and the reverse primer contains an in frame SalI restriction site.

Two separate PCR reactions were set up using 5 ng human testis and fetal brain cDNA templates, respectively. The reaction mixtures contained 1 microM of each of the 3352358CForward and 3352358CReverse primers, 5 micromoles dNTP (Clontech Laboratories, Palo Alto CA) and 1 10 microliter of 50xAdvantage-HF 2 polymerase (Clontech Laboratories, Palo Alto CA) in 50 microliter volume. The following reaction conditions were used:

a) 96°C 3 minutes

b) 96°C 30 seconds denaturation

c) 70°C 30 seconds, primer annealing. This temperature was gradually decreased by

15 1°C/cycle

d) 72°C 3 minutes extension.

Repeat steps (b)-(d) 10 times

e) 96°C 30 seconds denaturation

f) 60°C 30 seconds annealing

20 g) 72°C 3 minutes extension

Repeat steps (e)-(g) 25 times

h) 72°C 10 minutes final extension

The expected amplified product of 1335 bp was detected by agarose gel electrophoresis in both samples. The fragments were purified from agarose gel and ligated to pCR2.1 vector (Invitrogen, 25 Carlsbad, CA). Using M13 Forward and M13 Reverse vector primers and the following gene specific primers:

3352358 Seq-1 GTGCAGTAACCAGTTCAGCA (SEQ ID NO:46),

3352358 Seq-2 ACCTGTCCAAGCTGCGGGAG (SEQ ID NO:47),

3352358 Seq-3 TTGACGGGCTGGCTTCACTT (SEQ ID NO:48),

30 3352358 Seq-4 GACAGTGCTCAGCCACGCCT (SEQ ID NO:49),

the cloned insert was sequenced as PCR amplicons and verified as an open reading frame designated as 3352358-S153A. The nucleotide sequence (SEQ ID NO:31) obtained for this clone is shown in FIG. 17 Panel A. The cloning sites are in underlined italic font. The sequence obtained for clone 3352358-S153A differs from the sequence expected for clone 3352358-1 at six positions. These are 35 indicated in FIG. 17A by underlined bold font. The translated protein sequence (SEQ ID NO:32) for

clone 3352358-S153A is given in FIG. 17 Panel B. Five of the sequence differences found at the nucleotide level are translated into amino acid differences, compared to the sequence expected for clone 3352358-1; these are likewise indicated in FIG. 17B by underlined bold font. (In FIG. 17B, the two amino acid residues encoded by the cloning sites at each end are not shown. The first amino acid residue of FIG. 17B is encoded by nucleotides 7-9 of FIG. 17A.)

Example 5. Expression of h3352358 in human embryonic kidney 293 cells.

The vector pCEP4/Sec was prepared as described in Example 3. A 1.3 kb fragment containing the h3352358 sequence was isolated from pCR2.1-3352358 (prepared in Example 4) by BamHI-SalI digestion and subcloned into BamHI-XhoI digested pCEP4/Sec to generate the expression vector pCEP4/Sec-3352358. The pCEP4/Sec-3352358 vector was transfected into 293 cells using the LipofectaminePlus™ reagent following the manufacturer's instructions (Gibco/BRL). The cell pellet and supernatant were harvested 72 hours after transfection and examined for h3352358 expression by Western blotting (reducing conditions) with an anti-V5 antibody. FIG. 18 shows that h3352358 secreted by 293 cells is detected in a band carrying the V5 epitope at about 98 kDa. This band is presumed to represent the glycosylated form of the protein, since the program PROSITE predicts eight N-glycosylation sites in the extracellular h3352358 domain polypeptide.

Example 6. Expression of 2777610 in Tissues Determined by TaqMan™ Analysis

The expression of 2777610 was evaluated by real time quantitative PCR in tissues indicated in Table 3, below. The numbering in column 1 of Table 3 corresponds to the lane order of the histograms in FIGS 19A-C through FIG. 23.

Table 3. Panel of cell types used in TaqMan™ Analysis

1	Endothelial cells
2	Endothelial cells (treated)
3	Pancreas
4	Pancreatic ca. CAPAN 2
5	Adipose
6	Adrenal gland
7	Thyroid
8	Salivary gland
9	Pituitary gland
10	Brain (fetal)
11	Brain (whole)
12	Brain (amygdala)
13	Brain (cerebellum)
14	Brain (hippocampus)
15	Brain (hypothalamus)
16	Brain (substantia nigra)
17	Brain (thalamus)
18	Spinal cord
19	CNS ca. (glio/astro) U87-MG
20	CNS ca. (glio/astro) U-118-MG
21	CNS ca. (astro) SW1783

22 CNS ca.* (neuro; met) SK-N-AS
23 CNS ca. (astro) SF-539
24 CNS ca. (astro) SNB-75
25 CNS ca. (glial) SNB-19
26 CNS ca. (glial) U251
27 CNS ca. (glial) SF-295
28 Heart
29 Skeletal muscle
30 Bone marrow
31 Thymus
32 Spleen
33 Lymph node
34 Colon (ascending)
35 Stomach
36 Small intestine
37 Colon ca. SW480
38 Colon ca.* (SW480 met) SW620
39 Colon ca. HT29
40 Colon ca. HCT-116
41 Colon ca. CaCo-2
42 Colon ca. HCT-15
43 Colon ca. HCC-2998
44 Gastric ca.* (liver met) NCI-N87
45 Bladder
46 Trachea
47 Kidney
48 Kidney (fetal)
49 Renal ca. 786-0
50 Renal ca. A498
51 Renal ca. RXF 393
52 Renal ca. ACHN
53 Renal ca. UO-31
54 Renal ca. TK-10
55 Liver
56 Liver (fetal)
57 Liver ca. (hepatoblast) HepG2
58 Lung
59 Lung (fetal)
60 Lung ca. (small cell) LX-1
61 Lung ca. (small cell) NCI-H69
62 Lung ca. (s.cell var.) SHP-77
63 Lung ca. (large cell) NCI-H460
64 Lung ca. (non-sm. cell) A549
65 Lung ca. (non-s.cell) NCI-H23
66 Lung ca (non-s.cell) HOP-62
67 Lung ca. (non-s.cl) NCI-H522
68 Lung ca. (squam.) SW 900
69 Lung ca. (squam.) NCI-H596
70 Mammary gland
71 Breast ca.* (pl. effusion) MCF-7
72 Breast ca.* (pl.ef) MDA-MB-231
73 Breast ca.* (pl. effusion) T47D
74 Breast ca. BT-549

75	Breast ca.	MDA-N
76	Ovary	
77	Ovarian ca.	OVCAR-3
78	Ovarian ca.	OVCAR-4
79	Ovarian ca.	OVCAR-5
80	Ovarian ca.	OVCAR-8
81	Ovarian ca.	IGROV-1
82	Ovarian ca.* (ascites)	SK-OV-3
83	Myometrium	
84	Uterus	
85	Placenta	
86	Prostate	
87	Prostate ca.* (bone met)	PC-3
88	Testis	
89	Melanoma	Hs688(A).T
90	Melanoma* (met)	Hs688(B).T
91	Melanoma	UACC-62
92	Melanoma	M14
93	Melanoma	LOX IMVI
94	Melanoma* (met)	SK-MEL-5
95	Melanoma	SK-MEL-28
96	Melanoma	UACC-257

In the PCR assay used, a fluorogenic probe, consisting of an oligonucleotide with both a reporter and a quencher dye attached, is annealed specifically to the target sequence between the forward and reverse primers. When the probe is cleaved by the 5' nuclease activity of the DNA polymerase, the reporter dye is separated from the quencher dye and a sequence-specific signal is generated. With each cycle, additional reporter dye molecules are cleaved from their respective probes, and the increase in fluorescence intensity is monitored during the PCR.

5 Probes and primers were designed according to Perkin Elmer Biosystem's *Primer Express* Software package (version I for Apple Computer's Macintosh Power PC) using the sequence of 10 2777610 as input. Default settings were used for reaction conditions and the following parameters were set before selecting primers: primer concentration = 250 nM, primer melting temperature (T_m) range = 58°-60° C, primer optimal T_m = 59° C, maximum primer difference = 2° C, probe does not have 5' G, probe T_m must be 10° C greater than primer T_m , amplicon size 75 bp to 100 bp. The probes 15 and primers selected (see below) were synthesized, double HPLC purified to remove uncoupled dye and evaluated by mass spectroscopy for efficient coupling of reporter and quencher dyes to the 5' and 3' ends of the probe, respectively.

PCR conditions: Sample RNA was provided from a broad range of normal and tumor tissues. The RNA from each tissue (poly A+ RNA, 2.8 pg) and from the cell lines (total RNA, 70 ng) was spotted in each well of a 96 well PCR plate. PCR cocktails including the forward primer, reverse 20 primer and a 2777610-specific probe (see below; and another set of primers and a probe for another gene to serve as a reference) were set up using 1X TaqMan™ PCR Master Mix for the PE Biosystems

7700, with 5 mM MgCl₂, dNTPs (dA, dG, dC, dU at 1:1:1:2 ratios), 0.25 U/ml AmpliTaq Gold™ (PE Biosystems), and 0.4 U/μl RNase inhibitor, and 0.25 U/μl reverse transcriptase. Reverse transcription was performed at 48° C for 30 minutes followed by amplification/PCR cycles as follows: 95° C 10 min, then 40 cycles of 95° C for 15 seconds, 60° C for 1 minute.

5 TaqMan Probes and Primers Used in Analysis:

Ag 111 (F): 5'-CCTTTCAAAATCCTCTCTGACTCAC-3' (SEQ ID NO:50)

Ag 111 (R): 5'-TCACCGAAGAAAAACGACACAC-3' (SEQ ID NO:51)

Ag 111 (P): TET-5'-CCTGGCACCTGGCAGCTCAGA-3'-TAMRA (SEQ ID NO:52)

Example 7. Expression of 2864933 in Tissues Determined by TaqMan™ Analysis

TaqMan™ analysis of the expression of 2864933 was carried out as described in Example 6. Reverse transcription was performed at 48° C for 30 minutes followed by amplification/PCR cycles as follows: 95° C 10 min, then 40 cycles of 95° C for 15 seconds, 60° C for 1 minute. Primer-probe sets are as described below. The results are shown in FIG. 19, Panels A, B, and C, respectively. Cell types for each panel in FIG. 19 are as provided in Table 3, above.

15 Two sets of primers and a probe targeted regions of the nucleic acids that are common to
2864933-1 and 2864933-2. Primer-probe set 88 includes Ag 88 (SEQ ID NOs:53-55) and primer-
probe set 291 includes Ag 291(SEQ ID NOs:56-58).

Ag 88 (F): 5'-CATCTTCAACAGGCCATGGTT-3' (SEQ ID NO:53)

Ag 88 (R): 5'-AGCAGCTGTGTCAC TGCAA-3' (SEQ ID NO:54)

20 Ag 88 (P): TET-5'-TGAGAACAAATGGTCAGATAACCGCCTTACCAA-3'-TAMRA
(SEQ ID NO:55)

Ag 291 (F): 5'-CGCAGTCATTACCGGAGTCTT-3' (SEQ ID NO:56)

Ag 291 (R): 5'-TTCTTCAACCATTTGAATCGTG-3' (SEQ ID NO:57)

Ag 291 (P): TET-5'-AGCCCTACCCCTGCGGACCGTCA-3'-TAMRA (SEQ ID NO:58)

25 A third set of primers and a probe targeted the segment that is specifically present only in the longer splice variant, 2864933-1. Primer-probe set 341 includes Ag 341 (SEQ ID NOs:59-61).

Ag 341 (F): 5'-TCCTTTGTGGCACTGAATGG-3' (SEQ ID NO:59)

Ag 341 (R): 5'-CCCTCTT~~G~~AGCCGTCGAA-3' (SEQ ID NO:60)

Ag 341 (P): FAM-5'-TCCCTCTTGCCCCAGCACAAACCA-3'-TAMRA (SEQ ID NO:61)

30 Example 8. Expression of 3352358 in Tissues Determined by TaqMan™ Analysis

TaqMan™ analysis of the expression of 3352358 was carried out as described in Example 6, using the tissue panel as described in Table 3. Reverse transcription was performed at 48° C for 30 minutes followed by amplification/PCR cycles as follows: 95° C 10 min, then 40 cycles of 95° C for 15 seconds, 60° C for one (1) minute.

TaqMan™ probes and primers used in Analysis of 3352358 are shown below (primer and probe annealing positions to sequence input shown in color and underline respectively). Primer-probe set 42 includes Ag 42 (SEQ ID NOs:62-64). The results are shown in Fig. 20.

- 5 Ag 42 (F): 5'-CGCGAAAGTACAAGCCTGTTC-3' (SEQ ID NO:62)
Ag 42 (R): 5'-GAATGAGCACCGTGGTAGAGG-3' (SEQ ID NO:63)
Ag 42 (P): TET-5'-CGTCCACTGGTTACCAGCCGGCATATA-3'-TAMRA (SEQ ID NO:64)

Example 9. Expression of 3911675 in Tissues Determined by TaqMan™ Analysis

TaqMan™ analysis of the expression of 3911675 was carried out as described in Example 6, using the tissue panel as described in Table 3. Reverse transcription was performed at 48° C for 30 minutes followed by amplification/PCR cycles as follows: 95° C 10 min, then 40 cycles of 95° C for 15 seconds, 60° C for 1 minute. TaqMan Probes and Primers Used in the expression Analysis of 3911675 are the primer-probe set 115, which includes Ag 115 (SEQ ID NOs:65-67). The results are shown in Fig. 21.

- 15 Ag 115 (F): 5'-TGGACTCATCCCACTTGCTCT-3' (SEQ ID NO:65)
Ag 115 (R): 5'-CCTGCGCAAAAAGTTGTGAA-3' (SEQ ID NO:66)
Ag 115 (P): TET-5'-CAGCTGAATCCTGACATCATATCCACACTGTGT-3'-TAMRA
(SEQ ID NO:67)

Example 10. Expression of 4035508 in Tissues Determined by TaqMan™ Analysis

TaqMan™ analysis of the expression of 4035508 was carried out as described in Example 6,
 20 using the tissue panel as described in Table 3. Reverse transcription was performed at 48° C for 30
 minutes followed by amplification/PCR cycles as follows: 95° C 10 min, then 40 cycles of 95° C for
 15 seconds, 60° C for 1 minute. TaqMan probes and primers used in the expression analysis for clone
 4035508 include the primer-probe set 118, termed Ag 118 (SEQ ID NOS:68-70). The results are
 shown in Fig. 22.

- 25 Ag 118 (F): 5'-TCTCTGTCTGCAGTACCTGGCAT-3' (SEQ ID NO:68)
Ag 118 (R): 5'-GGCAGTGGGTATGGGATGTG-3' (SEQ ID NO:69)
Ag 118 (P): FAM-5'-ACTTCCCTCCTGATGCCCGGG-3'-TAMRA (SEQ ID NO:70)

Example 11. Expression of 4339264 in Tissues Determined by TaqMan™ Analysis

TaqMan™ analysis of the expression of 4339264 was carried out as described in Example 6, using the tissue panel as described in Table 3. Reverse transcription was performed at 48° C for 30 minutes followed by amplification/PCR cycles as follows: 95° C 10 min, then 40 cycles of 95° C for 15 seconds, 60° C for 1 minute. TaqMan probes and primers used in the expression analysis for clone 4339264 include the primer-probe set 120, termed Ag 120 (SEQ ID NOs:71-73). The results are shown in Fig. 23.

- 35 Ag 120 (F): 5'-AAAGGCGGAGGAAAGAAGTACTC-3' (SEQ ID NO:71)

Ag 120 (R): 5'-GCTCCCGTTCCCTCTCCA-3' (SEQ ID NO: 72)

Ag 120 (P): FAM-5'-CCTCTTGTCTTCTGCCGAGTTTCTT-3'-TAMRA (SEQ ID NO: 73)

EQUIVALENTS

5 From the foregoing detailed description of the specific embodiments of the invention, it should be apparent that unique nucleotides, polypeptides, and methods of use thereof for the SECX genes have been described. Although particular embodiments have been disclosed herein in detail, this has been done by way of example for purposes of illustration only, and is not intended to be limiting with respect to the scope of the appended claims which follow. In particular, it is contemplated by the
10 inventor that various substitutions, alterations, and modifications may be made to the invention without departing from the spirit and scope of the invention as defined by the claims. For instance, the choice of which SECX nucleotide or polypeptide or method of use thereof is believed to be a matter of routine for a person of ordinary skill in the art with knowledge of the embodiments described herein.

What is claimed is:

1. An isolated nucleic acid molecule encoding SECX, said molecule comprising a nucleotide sequence encoding a polypeptide having a sequence that is at least 99% identical to SEQ ID NOs:2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22, 24, 26, 28, 30, 32, 75, 77, 79 and 81, or the complement of said nucleic acid molecule.
2. The nucleic acid molecule of claim 1, wherein said nucleotide sequence encodes a polypeptide of SEQ ID NOs:2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22, 24, 26, 28, 30, 32, 75, 77, 79 and 81, or the complement of said nucleic acid molecule.
3. The nucleic acid molecule of claim 1, said molecule encoding the human SECX of SEQ ID NOs:1, 3, 5, 7, 9, 11, 13, 15, 17, 19, 21, 23, 25, 27, 29, 31, 74, 76, 78 and 80, or the complement of said nucleic acid molecule.
4. The isolated nucleic acid molecule of claim 1, said molecule hybridizing under stringent conditions to a nucleic acid sequence complementary to a nucleic acid molecule comprising the sequence of nucleotides of SEQ ID NOs:1, 3, 5, 7, 9, 11, 13, 15, 17, 19, 21, 23, 25, 27, 29, 31, 74, 76, 78 and 80, or the complement of said nucleic acid molecule.
5. The isolated nucleic acid molecule of claim 1, said molecule encoding a polypeptide comprising the amino acid sequence of SEQ ID NOs:2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22, 24, 26, 28, 30, 32, 75, 77, 79 and 81, or an amino acid sequence comprising one or more conservative substitutions in the amino acid sequence of SEQ ID NOs:2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22, 24, 26, 28, 30, 32, 75, 77, 79 and 81.
6. An oligonucleotide of the nucleic acid molecule of claim 1, said nucleic acid molecule less than 100 nucleotides in length and comprising at least 6 contiguous nucleotides of SEQ ID NOs:1, 3, 5, 7, 9, 11, 13, 15, 17, 19, 21, 23, 25, 27, 29, 31, 74, 76, 78 and 80, or a complement thereof.
7. A nucleic acid vector comprising the nucleic acid molecule of claim 1.
8. The nucleic acid vector of claim 7, wherein said vector is an expression vector.
9. The vector of claim 7, further comprising a regulatory element operably linked to said nucleic acid molecule.

10. A host cell comprising the isolated nucleic acid molecule of claim 1.
11. An isolated polypeptide at least 99% identical to a polypeptide selected from the group consisting of:
 - a) a polypeptide comprising an amino acid sequence of SEQ ID NOs:2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22, 24, 26, 28, 30, 32, 75, 77, 79 and 81;
 - b) a fragment of a polypeptide comprising an amino acid sequence of SEQ ID NOs:2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22, 24, 26, 28, 30, 32, 75, 77, 79 and 81, wherein the fragment comprises at least 6 contiguous amino acids of SEQ ID NOs:2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22, 24, 26, 28, 30, 32, 75, 77, 79 and 81;
 - c) a derivative of a polypeptide comprising an amino acid sequence of SEQ ID NOs:2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22, 24, 26, 28, 30, 32, 75, 77, 79 and 81;
 - d) an analog of a polypeptide comprising an amino acid sequence of SEQ ID NOs:2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22, 24, 26, 28, 30, 32, 75, 77, 79 and 81;
 - e) a homolog of a polypeptide comprising an amino acid sequence of SEQ ID NOs:2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22, 24, 26, 28, 30, 32, 75, 77, 79 and 81; and
 - f) a naturally occurring allelic variant of a polypeptide comprising an amino acid sequence of SEQ ID NOs:2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22, 24, 26, 28, 30, 32, 75, 77, 79 and 81, wherein the polypeptide is encoded by a nucleic acid molecule that hybridizes to a nucleic acid molecule c under stringent conditions.
12. The polypeptide of claim 11, wherein the polypeptide, or fragment thereof, comprises the amino acid sequence of at least one of SEQ ID NOs:2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22, 24, 26, 28, 30, 32, 75, 77, 79 and 81.
13. An antibody that selectively binds to the polypeptide of claim 11, and fragments, homologs, analogs, and derivatives of said antibody.
14. A method of producing the polypeptide of claim 11, said method comprising the step of culturing the host cell of claim 10 under conditions in which the nucleic acid molecule is expressed.
15. A method of detecting the presence of the polypeptide of claim 11 in a sample, comprising contacting the sample with a compound that selectively binds to the polypeptide of claim

11 and determining whether the compound bound to the polypeptide of claim 11 is present in the sample.

16. A method of detecting the presence of a nucleic acid molecule of claim 1 in a sample, the method comprising contacting the sample with a nucleic acid probe or primer that selectively binds to the nucleic acid molecule and determining whether the nucleic acid probe or primer bound to the nucleic acid molecule of claim 1 is present in the sample.

17. A method for modulating the activity of the polypeptide of claim 11, the method comprising contacting a cell sample comprising the polypeptide of claim 11 with a compound that binds to said polypeptide in an amount sufficient to modulate the activity of the polypeptide.

18. A method of treating or preventing a SECX-associated disorder, said method comprising administering to a subject in which such treatment or prevention is desired an amount of a therapeutic selected from the group consisting of:

- a) the nucleic acid of claim 1;
- b) the polypeptide of claim 11; and
- c) the antibody of claim 13;

wherein said therapeutic is administered in an amount sufficient to treat or prevent said SECX-associated disorder in said subject.

19. A pharmaceutical composition comprising a therapeutically or prophylactically effective amount of a therapeutic selected from the group consisting of:

- a) the nucleic acid of claim 1;
- b) the polypeptide of claim 11; and
- c) the antibody of claim 13;

and a pharmaceutically acceptable carrier.

20. A kit comprising in one or more containers, a therapeutically or prophylactically effective amount of the pharmaceutical composition of claim 19.

21. The use of a therapeutic in the manufacture of a medicament for treating a syndrome associated with a human disease, the disease selected from a SECX-associated disorder, wherein said therapeutic is selected from the group consisting of:

- a) the nucleic acid of claim 1;

- b) the polypeptide of claim 11; and
- c) the antibody of claim 13.

22. A method for screening for a modulator of activity or of latency or predisposition to a SECX-associated disorder, said method comprising:

- a) administering a test compound to a test animal at increased risk for a SECX-associated disorder, wherein said test animal recombinantly expresses a SECX protein;
- b) measuring expression the activity of said protein in said test animal;
- c) measuring the activity of said protein in a control animal that recombinantly expresses said protein and is not at increased risk for a SECX-associated disorder; and
- d) comparing expression of said protein in said test animal and said control animal, wherein a change in the activity of said protein in said test animal relative to said control animal indicates the test compound is a modulator of latency of a SECX-associated disorder.

23. The method of claim 22, wherein said test animal is a recombinant test animal that expresses a test protein transgene or expresses said transgene under the control of a promoter at an increased level relative to a wild-type test animal, and wherein said promoter is not the native gene promoter of said transgene.

24. A method for determining the presence of or predisposition to a disease associated with altered levels of a SECX polypeptide of claim 11, the method comprising:

- a) measuring the amount of the polypeptide in a sample from the mammalian subject; and
- b) comparing the amount of said polypeptide in step (a) to the amount of the polypeptide present in a control sample,

wherein an alteration in the level of the polypeptide in step (a) as compared to the control sample indicates a disease condition.

25. A method for determining the presence of or predisposition to a disease associated with altered levels of a SECX nucleic acid of claim 1, the method comprising:

- a) measuring the amount of the nucleic acid in a sample from the mammalian subject; and

- b) comparing the amount of said nucleic acid in step (a) to the amount of the nucleic acid present in a control sample,

wherein an alteration in the level of the nucleic acid in step (a) as compared to the control sample indicates a disease condition.

26. A method of treating a pathological state in a mammal, the method comprising administering to the subject a polypeptide to a subject in an amount to alleviate the pathological condition, wherein the polypeptide a polypeptide having an amino acid sequence at least 95% identical to a polypeptide with an amino acid sequence of SEQ ID NOS:2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22, 24, 26, 28, 30, 32, 75, 77, 79 and 81, or a biologically active fragment thereof.

27. A method of treating a pathological state in a mammal, the method comprising administering to the subject the antibody of claim 13 in an amount sufficient to alleviate the pathological condition.

Figure 1. Clone 2777610

ATGACTGCCGCCATAAGAACAGAGAGAACTGAGTATCCTCCAAAGGTGAATTCAAT
 TTTGTTATTATGAGTGTGCTGTTATATAAAAAGAATATGCTTAAGGGAAAAAGGTGC
 TTTAAAGTTAATATTCTACAAACCATAGTTATGAGCATAAGAAATTACATAATTACAG
 CAATCTGATGTATTAGTAATAATAATGTATTATTCTCTTAAACAGTGTGTTTAT
 GGCTAACAGTAGCACCTGTGAATGAGGCAGAACCTGTATTGGATTCACAAGGATGTG
 AAAGTAATGGTACTGTTAAAGTACCAAAATGTATTATGCTTAAAGTAGCC
 GAAAACAGTATTTCTTCAACACATCTATTGAAAGTGTGATAATGCAGGATGTT
 AATATGCTATAAACATAAAAGTCTGTTTAAAGTAGCATTTGAAAATCATGAAGGGCT
 TTTGTTTCTTGTATATGTTATTGGTAAAGGTGACACTGGAAGCAATGA
MetAsn
 ACACCACAGTGATGCAAGGCTCAACAGATCTGAGCGGTGCCAGAGACACTCGGATAG
 ThrThrValMetGlnGlyPheAsnArgSerGluArgCysProArgAspThrArgIleVal
 TACAGCTGGTATTCCCAGCCCTACACAGTGGTTTCTTGACCGGCATCTGCTGAATA
 GlnLeuValPheProAlaLeuTyrThrValValPheLeuThrGlyIleLeuLeuAsnThr
 CTTGGCTCTGGGGTGTTCACATCCCCAGCTCCTCCACCTTCATCATCTACCTCA
 LeuAlaLeuTrpValPheValHisIleProSerSerThrPheIleIleTyrLeuLys
 AAAACACTTTGGTGGCCGACTTGATAATGACACTCATGCTTCCTTCAAAATCCTCTG
 AsnThrLeuValAlaAspLeuIleMetThrLeuMetLeuProPheLysIleLeuSerAsp
 ACTCACACCTGGCACCTGGCAGCTCAGAGCTTTGTGTGCTGTTCTCGGTGATAT
 SerHisLeuAlaProTrpGlnLeuArgAlaPheValCysArgPheSerSerValIlePhe
 TTATGAGACCATGTATGAGGTCATCGTGTGTTAGGGCTCATAGCCTTGACAGATTCC
 TyrGluThrMetTyrValGlyIleValLeuLeuGlyLeuIleAlaPheAspArgPheLeu
 TCAAGATCATCAGACCTTGAGAAATTTCTAAAAAAACCTGTTTGCAAAACGG
 LysIleIleArgProLeuArgAsnIlePheLeuLysLysProValPheAlaLysThrVal
 TCTCAATCTCATCTGGTCTTTGTTCTTCATCTCCCTGCCAAATATGATCTGAGCA
 SerIlePheIleTrpPhePheLeuPhePheIleSerLeuProAsnMetIleLeuSerAsn
 ACAAGGAAGCAACACCATCGTGTGAAAAAGCTGCTGCTTAAAGGGCCTCTGGGG
 LysGluAlaThrProSerSerValLysLysCysAlaSerLeuLysGlyProLeuGlyLeu
 TGAAATGGCATCAAATGGTAAATAACATATGCCAGTTATTTCTGGACTGTTTATCC
 LysTrpHisGlnMetValAsnAsnIleCysGlnPheIlePheTrpThrValPheIleLeu
 TAATGCTTGTGTTTATGAGTGTGTTATGCAAAAAAGTATATGATTCTTATAGAAAGTCCA
 MetLeuValPheTyrValValIleAlaLysLysValTyrAspSerTyrArgLysSerLys
 AAAGTAAGGACAGAAAAAACAAAAAGCTGGAAGGCAAAGTATTGTTGCTGTTGCTG
 SerLysAspArgLysAsnAsnLysLysLeuGluGlyLysValPheValValAlaVal
 TCTTCTTGTGTTGCTCCATTCTTTGCCAGAGTCCATATACTCACAGTC
 PhePheValCysPheAlaProPheHisPheAlaArgValProTyrThrHisSerGlnThr
 CCAACAATAAGACTGACTGTAGACTGCAAAATCAACTGTTATTGCTAAAGAAACAACTC
 AsnAsnLysThrAspCysArgLeuGlnAsnGlnLeuPheIleAlaLysGluThrThrLeu
 TCTTTGGCAGCAACTAACATTGTATGGATCCCTTAATATACATATTCTTATGTAAAA
 PheLeuAlaAlaThrAsnIleCysMetAspProLeuIleTyrIlePheLeuCysLysLys

AATTCAAGAAAAGCTACCATGTATGCAAGGGAGAAAGACCACAGCATCAAGCCAAGAAA
PheThrGluLysLeuProCysMetGlnGlyArgLysThrThrAlaSerSerGlnGluAsn

ATCATAGCAGTCAGACAGAACATAACCTTAGGCTGACAACGTACATAGGGTTAACTT
HisSerSerGlnThrAspAsnIleThrLeuGly

CTATTTATTGATGAGACTCCGTAGATAATGTGGAAATCAAATTAAACCAAGAAAAAAAG

ATTGGAACAAATGCTCTTACATTTATTATCCTCGTGTACAGAAAAGATTATATAAAA

TTTAAATCCACATAGATCTATTCTAAAGCTGAATGAACCATTACTAAGAGAATGCAACAG

GATACAAATGCCACTAGAGGTCAATTATTCCTTCTTTATTCAAGCGGCCGCTTTTT

TTTTTTTTTTTT

Figure 2. Clone 2864933_1

GCGACTATTCCCCCAAAGAGACAAGCACACATGTAGGAATGACAAGGCTTGCAGAGGA
 GAGAGCGCAGCCCGGCCGGAGAGATCCCCTGATAATGGATTACTAAATGGATACA
 CGCTGTACCAGTCGCTCCGAGCCCCGGCCGCTGTCGATGCACCGAAAAGGGTGA
 AGTAGAGAAATAAGTCTCCCGCTGAACACTACTATGAGGTCAAAGCCTGCTGCTATAT
 MetArgSerGluAlaLeuLeuTyr
 TTCACACTGCTACACTTGCTGGGCTGGTTCCAGAAGATTCTGAGCCAATCAGTATT
 PheThrLeuLeuHisPheAlaGlyAlaGlyPheProGluAspSerGluProIleSerIle
 TCGCATGGCAACTATACAAAACAGTATCGGTGTTGTGGGCCACAAGCCAGGACGGAAC
 SerHisGlyAsnTyrThrLysGlnTyrProValPheValGlyHisLysProGlyArgAsn
 ACCACACAGAGGCACAGGCTGGACATCCAGATGATTATGATCATGAACGGAACCTCTAC
 ThrThrGlnArgHisArgLeuAspIleGlnMetIleMetIleMetAsnGlyThrLeuTyr
 ATTGCTGCTAGGGACCATAACTGTTGATATAGACACATCACACACGGAAAGAAATT
 IleAlaAlaArgAspHisIleTyrThrValAspIleAspThrSerHisThrGluGluIle
 TATTGTAGCAAAAAACTGACATGGAAATCTAGACAGGCCGATGTAGACACATGCAGAAC
 TyrCysSerLysLysLeuThrTrpLysSerArgGlnAlaAspValAspThrCysArgMet
 AAGGGAAAACATAAGGATGAGTGCCACAACCTTTATTAAAGTTCTCTAAAGAAAAACGAT
 LysGlyLysHisLysAspGluCysHisAsnPheIleLysValLeuLysLysAsnAsp
 GATGCATTGTTGCTGTGGAACTAATGCCTTCAACCCCTCCTGCAGAAACATATAAGATG
 AspAlaLeuPheValCysGlyThrAsnAlaPheAsnProSerCysArgAsnTyrLysMet
 GATACATTGGAACCATTCGGGATGAATTTCAGCGGAATGCCAGATGCCATATGATGCC
 AspThrLeuGluProPheGlyAspGluPheSerGlyMetAlaArgCysProTyrAspAla
 AAACATGCCAACGTTGCACTGTTGCAGATGGAAAACATATACTCAGCCACAGTGACTGAC
 LysHisAlaAsnValAlaLeuPheAlaAspGlyLysLeuTyrSerAlaThrValThrAsp
 TTCCATTGCCATTGACGCAGTCATTACCGGAGTCTGGAGAAAGCCCTACCTGGGACC
 PheLeuAlaIleAspAlaValIleTyrArgSerLeuGlyGluSerProThrLeuArgThr
 GTCAAGCACGATTCAAAATGTTGAAAGAACCATACTTGTCAAGCCGTGGATTACGGA
 ValLysHisAspSerLysTrpLeuLysGluProTyrPheValGlnAlaValAspTyrGly
 GATTATATCTACTTCTTCAGGGAAATAGCAGTGGAGTATAACACCATGGAAAGGTA
 AspTyrIleTyrPhePheArgGluIleAlaValGluTyrAsnThrMetGlyLysVal
 GTTTCCCAAGAGTGGCTCAGGTTGTAAGAATGATATGGGAGGATCTCAAAGAGTCCTG
 ValPheProArgValAlaGlnValCysLysAsnAspMetGlyGlySerGlnArgValLeu
 GAGAAACAGTGGACGTCGTTCTGAAGGCGCGCTGAACTGCTCAGTTCTGGAGACTCT
 GluLysGlnTrpThrSerPheLeuLysAlaArgLeuAsnCysSerValProGlyAspSer
 CATTATTCACATTCTCCAGGCAGTTACAGATGTGATTCTGATCAACGGCGTGAT
 HisPheTyrPheAsnIleLeuGlnAlaValThrAspValIleArgIleAsnGlyArgAsp
 GTTGCCTGGCAACGTTTACACCTTATAACAGCATCCCTGGGTCTGCAGTCTGTGCC
 ValValLeuAlaThrPheSerThrProTyrAsnSerIleProGlySerAlaValCysAla
 TATGACATGCTTGACATTGCCAGTGTGTTTACTGGGAGATTCAAGGAACAGAAGTCTCCT
 TyrAspMetLeuAspIleAlaSerValPheThrGlyArgGlySerGlnLysSerPro

GATTCCACCTGGACACCAGTTCTGATGAACGAGTCTCTAAGCCAGGCCAGGTTGCTGT
AspSerThrTrpThrProValProAspGluArgValProLysProArgProGlyCysCys

GCTGGCTCATCCTCCTTAGAAAGATATGCAACCTCCAATGAGTCCCTGATGATAACCTG
AlaGlySerSerSerLeuGluArgTyrAlaThrSerAsnGluPheProAspAspThrLeu

AACTTCATCAAGACGCACCCGCTCATGGATGAGGCAGTGCCTCCATCTCACAGGCCA
AsnPheIleLysThrHisProLeuMetAspGluAlaValProSerIlePheAsnArgPro

TGGTTCTGAGAACAAATGGTCAGATACCGCCTTACCAAAATTGCACTGGACACAGCTGCT
TrpPheLeuArgThrMetValArgTyrArgLeuThrLysIleAlaValAspThrAlaAla

GGGCCATATCAGAACACTGTGGTTTCTGGATCAGAGAAGGGAATCATCTTGAAAG
GlyProTyrGlnAsnHisThrValValPheLeuGlySerGluLysGlyIleIleLeuLys

TTTTTGGCCAGAACATAGGAAATAGTGGTTTCTAAATGACAGCCTTCCTGGAGGAGATG
PheLeuAlaArgIleGlyAsnSerGlyPheLeuAsnAspSerLeuPheLeuGluGluMet

AGTGTTCACAACCTGAAAATGCAGCTATGATGGAGTCGAAGAACAAAAGGATCATGGGC
SerValTyrAsnSerGluLysCysSerTyrAspGlyValGluAspLysArgIleMetGly

ATGCAGCTGGACAGAGCAAGCAGCTCTGTATGTTGCGTTCTCACCTGTGTGATAAAG
MetGlnLeuAspArgAlaSerSerLeuTyrValAlaPheSerThrCysValIleLys

GTTCCCCTGGCCGGTGTGAACGACATGGAAAGTGTAAAAAAACCTGTATTGCCTCCAGA
ValProLeuGlyArgCysGluArgHisGlyLysCysLysLysThrCysIleAlaSerArg

GACCCATATTGGATGGATAAAGGAAGGTGGTGCCTGCAGCCATTATCACCAACAGC
AspProTyrCysGlyTrpIleLysGluGlyAlaCysSerHisLeuSerProAsnSer

AGACTGACTTTGAGCAGGACATAGAGCGTGGCAATACAGATGGCTGGGGACTGTCAC
ArgLeuThrPheGluGlnAspIleGluArgGlyAsnThrAspGlyLeuGlyAspCysHis

AATTCCCTTGCGACTGAATGGCATTCCAGTCCCTCTGCCAGCACAAACACATCA
AsnSerPheValAlaLeuAsnGlyHisSerSerSerLeuLeuProSerThrThrSer

GATTGACGGCTCAAGAGGGGTATGAGTCTAGGGAGGAATGCTGGACTGGAAGCATCTG
AspSerThrAlaGlnGluGlyTyrGluSerArgGlyGlyMetLeuAspTrpLysHisLeu

CTTGACTCACCTGACAGCACAGACCCCTTGGGGCGAGTGTCTCCCATAATCACCAAGAC
LeuAspSerProAspSerThrAspProLeuGlyAlaValSerHisAsnHisGlnAsp

AAGAAGGGAGTGATTGGAAAGTTACCTCAAAGGCCACGACCAGCTGGTCCCGTCACC
LysLysGlyValIleArgGluSerTyrLeuLysGlyHisAspGlnLeuValProValThr

CTCTTGGCCATTGCAGTCATCCTGGCTTCGTATGGGGCCGTCTCTGGCATCACC
LeuLeuAlaIleAlaValIleLeuAlaPheValMetGlyAlaValPheSerGlyIleThr

GTCTACTGCGTCTGTGATCATCGCGCAAAGACGCTGGCTGTGGTGCAGCGCAAGGAGAAG
ValTyrCysValCysAspHisArgArgLysAspValAlaValValGlnArgLysGluLys

GAGCTCACCCACTCGGCCGGGGCTCCATGAGCAGCGTACCAAGCTCAGCGGCCTTT
GluLeuThrHisSerArgArgGlySerMetSerSerValThrLysLeuSerGlyLeuPhe

GGGGACACTCAATCAAAGACCCAAAGCCGGAGGCCATCCTCACGCCACTCATGCACAAAC
GlyAspThrGlnSerLysAspProLysProGluAlaIleLeuThrProLeuMetHisAsn

GGCAAGCTGCCACTCCCGCAACACGCCAGATGCTCATTAAAGCAGACCAGCACCAC
GlyLysLeuAlaThrProGlyAsnThrAlaLysMetLeuIleLysAlaAspGlnHisHis

CTGGACCTGACGGCCCTCCCCACCCAGAGTCACCCCAACGCTGCAAGCAGGGAA
LeuAspLeuThrAlaLeuProThrProGluSerThrProThrLeuGlnGlnLysArgGlu

CCCAGCCGGGACCGCGAGTGGAGAGGAACCAGAACCTCATCAATGCCGCACAAAG
ProSerArgGlyThrArgGluTrpGluArgAsnGlnAsnLeuIleAsnAlaCysThrLys

GACATGCCCTCATGGGCTCCCCTGTGATTCCCACGGACCTGCCCTGCCGGCTCCCC
AspMetProProMetGlySerProValIleProThrAspLeuProLeuArgAlaSerPro

AGCACATCCCCAGCGTGGTGGTCCTGCCATCACCGAGCAGGGCTACCAGCATGAGTAC
SerHisIleProSerValValValLeuProIleThrGlnGlnGlyTyrGlnHisGluTyr

GTGGACCAGCCAAAATGAGCGAGGTGGCCCAGATGGCCTGGAGGACCAGGCCACA
ValAspGlnProLysMetSerGluValAlaGlnMetAlaLeuGluAspGlnAlaAlaThr

CTGGAGTATAAGACCATCAAGGAACATCTCAGCAGCAAGAGTCCAACCATGGGTGAAC
LeuGluTyrLysThrIleLysGluHisLeuSerSerLysSerProAsnHisGlyValAsn

CTTGTGGAGAACCTGGACAGCCTGCCCTAAAGTTCCACAGCGGAGGCCTCCCTGGGT
LeuValGluAsnLeuAspSerLeuProProLysValProGlnArgGluAlaSerLeuGly

CCCCGGGAGCCTCCCTGTCTCAGACCGTCAAGCAAGCGGCTGAAATGCACCACTCC
ProProGlyAlaSerLeuSerGlnThrGlyLeuSerLysArgLeuGluMetHisHisSer

TCTTCTACGGGTTGACTATAAGAGGAGCTACCCCACGAACCGCTACGAGAAGGCCAC
SerSerTyrGlyValAspTyrLysArgSerTyrProThrAsnSerLeuThrArgSerHis

CTGACCACCTACTCTCATCAGAACACTAACCCGACAATTCANCTCTGACTTC
LeuThrThrTyrSerHisGlnLysGlnHis

GGGACCAGAGCTTGGCAGGGAGACAACCCGCCGCCGAGGGTGGACTCCA

TCCAGGTGCACAGCTCCAGCCATCTGCCAGGCCGTGACTGTCTCGAGGCAGGCCAGCC

TCAACGCCTACAACACTCACTGACAAGGTGGGCTGAAGCGTACGCCCTCGCTAAAGCCGG

ACGTACCCCCCAAACCATCCTTGCTCCCTTCCACATCCATGAAGCCAATGATGCGT

GTACATAATCCAGGGGGAGGGGTCAAGTGTGAAACAGCAGGCAAGGCGAGGTGTCCG

CTCAGCTCAGCAAGGTTCTCAACTGCCCTCGAGTACCCACCAACAAAAAGGCCCTGCC

AGAACCGAGGGACGCTGGGTCCCTCTGGGACACAGGGTACTCACGAAACTGGC

CGCGTGGTTGGTGAAAG

Figure 3. Clone 2864933-2

GCGACTATTCCCCAAAGAGACAAGCACACATGTAGGAATGACAAAGGCTTGCAGAGGA
 GAGAGCGCAGCCCGGGCCCGAGAGATCCCCTCGATAATGGATTACTAAATGGATACA
 CGCTGTACCAGTCGCTCCGAGCCCCGGCCCTGTCCGTCATGCACCGAAAAGGGTGA
 AGTAGAGAAATAAAGTCTCCCCGCTGAACACTATGAGGTAGAACGCCCTGCTGCTATAT
 MetArgSerGluAlaLeuLeuLeuTyr
 TTCACACTGCTACACTTGCTGGGCTGGTTCCAGAACAGATTCTGAGCCAATCAGTATT
 PheThrLeuLeuHisPheAlaGlyAlaGlyPheProGluAspSerGluProIleSerIle
 TCGCATGGCAACTATACAAAACAGTATCCGGTGTGTTGGGCCACAAGCCAGGACGGAAC
 SerHisGlyAsnTyrThrLysGlnTyrProValPheValGlyHisLysProGlyArgAsn
 ACCACACAGAGGCACAGGCTGGACATCCAGATGATTATGATCATGAACGGAACCCCTAC
 ThrThrGlnArgHisArgLeuAspIleGlnMetIleMetIleMetAsnGlyThrLeuTyr
 ATTGCTGCTAGGGACCATATTATACTGTTGATATAGACACATCACACACGGAAAGAAATT
 IleAlaAlaArgAspHisIleTyrThrValAspIleAspThrSerHisThrGluGluIle
 TATTGTAGAAAAAACTGACATGGAAATCTAGACAGGCCGATGTAGACACATGCAGAATG
 TyrCysSerLysLeuThrTrpLysSerArgGlnAlaAspValAspThrCysArgMet
 AAGGGAAAACATAAGGGATGAGTGCACAACTTTATTAAAGTTCTCTAAAGAAAAACGAT
 LysGlyLysHisLysAspGluCysHisAsnPheIleLysValLeuLeuLysLysAsnAsp
 GATGCATTGTTGTCTGTGGAACTAATGCCCTCAACCCCTCCTGCAGAAAATATAAGATG
 AspAlaLeuPheValCysGlyThrAsnAlaPheAsnProSerCysArgAsnTyrLysMet
 GATACATTGGAACCATTGGGGATGAATCAGCGGAATGCCAGATGCCATATGATGCC
 AspThrLeuGluProPheGlyAspGluPheSerGlyMetAlaArgCysProTyrAspAla
 AAACATGCCAACGTTGCACTGTTGCAGATGGAAAATCTACTCAGCCACAGTGACTGAC
 LysHisAlaAsnValAlaLeuPheAlaAspGlyLysLeuTyrSerAlaThrValThrAsp
 TTCCATTGCCATTGACGCAGTCATTACCGGAGTCTGGAGAAAGCCCTACCCCTGCGGACC
 PheLeuAlaIleAspAlaValIleTyrArgSerLeuGlyGluSerProThrLeuArgThr
 GTCAAGCACGATTCAAATGGTTGAAAGAACCATACTTGTCAAGCCGTGGATTACGGA
 ValLysHisAspSerLysTrpLeuLysGluProTyrPheValGlnAlaValAspTyrGly
 GATTATATCTACTTCTTCAGGGAAATAGCAGTGGAGTATAACACCATGGGAAAGGTA
 AspTyrIleTyrPhePhePheArgGluIleAlaValGluTyrAsnThrMetGlyLysVal
 GTTTCCCAAGAGTGGCTCAGGTTGTAAGAATGATATGGGAGGATCTCAAAGAGTCCTG
 ValPheProArgValAlaGlnValCysLysAsnAspMetGlyGlySerGlnArgValLeu
 GAGAAAACAGTGGACGCTCGTCTGAAGGCGCGCTGAACTGCTCAGTCTGGAGACTCT
 GluLysGlnTrpThrSerPheLeuLysAlaArgLeuAsnCysSerValProGlyAspSer
 CATTATTTATTCACATTCTCCAGGCAGTTACAGATGTGATTGATCAACGGCGGTGAT
 HisPheTyrPheAsnIleLeuGlnAlaValThrAspValIleArgIleAsnGlyArgAsp
 GTTGTCTGGCAACGTTCTACACCTTATAACAGCATCCCTGGGTCTGCAGTCTGTGCC
 ValValLeuAlaThrPheSerThrProTyrAsnSerIleProGlySerAlaValCysAla
 TATGACATGCTTGACATTGCCAGTGTGTTACTGGGAGATTCAAGGAACAGAAGTCTCCT
 TyrAspMetLeuAspIleAlaSerValPheThrGlyArgPheLysGluGlnLysSerPro

GATTCCACCTGGACACCAGTCCTGATGAACGAGTCTTAAGCCCAGGCCAGGTTGCTGT
 AspSerThrTrpThrProValProAspGluArgValProLysProArgProGlyCysCys

 GCTGGCTCATCCTCCTTAGAAAGATATGCACACCTCCAATGAGTCCCTGATGATAACCCCTG
 AlaGlySerSerLeuGluArgTyrAlaThrSerAsnGluPheProAspAspThrLeu

 AACTTCATCAAGACGCACCCGCTCATGGATGAGGCAGTGCCTCCATCTTCAACAGGCCA
 AsnPheIleLysThrHisProLeuMetAspGluAlaValProSerIlePheAsnArgPro

 TGGTTCCCTGAGAACAAATGGTCAGATAACCGCCTTACCAAAATTGAGTGGACACAGCTGCT
 TrpPheLeuArgThrMetValArgTyrArgLeuThrLysIleAlaValAspThrAlaAla

 GGGCCATATCAGAACATCACACTGTGGTTTCTGGGATCAGAGAAGGGAAATCATCTTGAAG
 GlyProTyrGlnAsnHisThrValValPheLeuGlySerGluLysGlyIleIleLeuLys

 TTTTG GCCAGAACATAGGAAATAGTGGTTTCTAAATGACAGCCTTCTGGAGGAGATG
 PheLeuAlaArgIleGlyAsnSerGlyPheLeuAsnAspSerLeuPheLeuGluGluMet

 AGTGTTCACAACTCTGAAAAATGAGCTATGATGGAGTCGAAGAACAAAGGATCATGGGC
 SerValTyrAsnSerGluLysCysSerTyrAspGlyValGluAspLysArgIleMetGly

 ATGCAGCTGGACAGAGCAAGCAGCTCTGTATGTTGCGTTCTACCTGTGTGATAAAG
 MetGlnLeuAspArgAlaSerSerLeuTyrValAlaPheSerThrCysValIleLys

 GTTCCCCCTGGCCGGTGTGAACGACATGGGAAGTGTAAAAAAACCTGTATTGCCCTCCAGA
 ValProLeuGlyArgCysGluArgHisGlyLysCysLysThrCysIleAlaSerArg

 GACCCATATTGTGGATGGATAAAGGAAGGTGGTGCCTGCAGCCATTATCACCCAACAGC
 AspProTyrCysGlyTrpIleLysGluGlyGlyAlaCysSerHisLeuSerProAsnSer

 AGACTGACTTTGAGCAGGACATAGAGCGTGGCAATACAGATGGCTGGGGACTGTCAC
 ArgLeuThrPheGluGlnAspIleGluArgGlyAsnThrAspGlyLeuGlyAspCysHis

 AATTCCCTTGCCACTGAATGGAGTGATTGGAAAGTTACCTCAAAGGCCACGACCAG
 AsnSerPheValAlaLeuAsnGlyValIleArgGluSerTyrLeuLysGlyHisAspGln

 CTGGTCCCGTACCCCTTGGCCATTGCGTCTGTGATCATCGGCGAAAGACGTGGCTGGTG
 LeuValProValThrLeuLeuAlaIleAlaValIleLeuAlaPheValMetGlyAlaVal

 TTCTCGGGCATTACCGTCACTGCGTCTGTGATCATCGGCGAAAGACGTGGCTGGTG
 PheSerGlyIleThrValTyrCysValCysAspHisArgArgLysAspValAlaValVal

 CAGCGCAAGGAGAAGGAGCTCACCACTCGGCCGGGCTCCATGAGCAGCGTCACCAAG
 GlnArgLysGluLysGluLeuThrHisSerArgArgGlySerMetSerSerValThrLys

 CTCAGCGGCCTTTGGGACACTCAATCAAAGACCCAAAGCCGGAGGCCATCCTCACG
 LeuSerGlyLeuPheGlyAspThrGlnSerLysAspProLysProGluAlaIleLeuThr

 CCACTCATGCACAACGGCAAGCTGCCACTCCGGCAACACGGCAAGATGCTCATTAAA
 ProLeuMetHisAsnGlyLysLeuAlaThrProGlyAsnThrAlaLysMetLeuIleLys

 GCAGACCAGCACCACTGGACCTGACGCCCTCCCCACCCAGAGTCAACCCAACGCTG
 AlaAspGlnHisHisLeuAspLeuThrAlaLeuProThrProGluSerThrProThrLeu

 CAGCAGAACGGGAAACCCAGCCGGCACCGCGAGTGGGAGAGGAACCAGAACCTCATC
 GlnGlnLysArgGluProSerArgGlyThrArgGluTrpGluArgAsnGlnAsnLeuIle

 AATGCCTGCACAAAGGACATGCCCATGGCTCCCTGTGATTCCCACGGACCTGCC
 AsnAlaCysThrLysAspMetProMetGlySerProValIleProThrAspLeuPro

 CTGCGGGCCTCCCCAGCCACATCCCCAGCGTGGTGGCCTGCCATACGCAGCAGGGC
 LeuArgAlaSerProSerHisIleProSerValValLeuProIleThrGlnGlnGly

TACCAGCATGAGTACGTGGACCAGCCAAAATGAGCGAGGTGGCCCAGATGGCGCTGGAG
TyrGlnHisGluTyrValAspGlnProLysMetSerGluValAlaGlnMetAlaLeuGlu

GACCAGGCCAACACTGGAGTATAAGACCATCAAGGAACATCTCAGCAGCAAGAGTCCC
AspGlnAlaAlaThrLeuGluTyrLysThrIleLysGluHisLeuSerSerPro

AACCATGGGGTGAACCTTGTGGAGAACCTGGACAGCCTGCCCTGTCTCAGACCGGTCTAAGCAAGCGGCTG
AsnHisGlyValAsnLeuValGluAsnLeuAspSerLeuProProLysValProGlnArg

GAGGCCTCCCTGGTCCCCGGGAGCCTCCCTGTCTCAGACCGGTCTAAGCAAGCGGCTG
GluAlaSerLeuGlyProProGlyAlaSerLeuSerGlnThrGlyLeuSerLysArgLeu

GAAATGCACCACTCCTCTTCTACGGGGTTGACTATAAGAGGAGCTACCCACGAACCTG
GluMetHisHisSerSerSerTyrGlyValAspTyrLysArgSerTyrProThrAsnSer

CTCACGAGAACCCACCTGACCACCTACTCTCATCAGAACACTAACCCGACAATTG
LeuThrArgSerHisLeuThrThrSerHisGlnLysGlnHis

ANCTCTGACTTCAAAGGGACCAGAGCTTGGCAGGGAGACAACCCGCCGCCGCCCCGC
AGAGGGTGGACTCCATCCAGGTGCACAGCTCCAGCCATCTGGCCAGGCCGTGACTGTCT

CGAGGCAGCCAGCCTAACGCCTACAACTCACTGACAAGGTCGGGCTGAAGCGTACGC
CCTCGCTAAAGCCGGACGTACCCCCAAACCATCCTTGCTCCCCTTCCACATCCATGA

AGCCAATGATGCGTGTACATAATCCCAGGGGAGGGGTCAGGTGTCGAACCAGCAGGC
AAGGCAGGTGTCGCTCAGCTCAGCAAGGTTCTCAACTGCCTCGAGTACCCACCAAACC
AAAAAGGCCTGGCGAGAACCGAGGGACGCTGGGTCTCCTCTGGGACACAGGGTAC
TCACGAAAATGGGCCGCGTGGTTGGTGAAAG

Figure 4. Clone 2982339

TCTCCCCTTCAGCTGAAAGGCTATTGTCATGAGATTAGAATTCCAGTCAACACTGGT
 ATTGGAAACTATTTGCAGTAGTAGACAAGGGAGTCGCAATCATTCATATCATTACATT
 TCTGTGTTTCTGTGATGATCATGAACATTGCTCAGAGCAATGCTGTGATATCACAGT
 Met Ile Met Asn Ile Ala Gln Ser Asn Ala Val Ile Ser Gln Trp
 GGCTATTTATGATTAGATCATTGATGCTTACACTTTATGGAAAAATGTAACA
 Leu Phe Met Ile Arg Ser Phe His Cys Met Leu Thr Leu Phe Met Glu Lys Cys Asn Lys
 AATGTCAAATATAATCAGAAATTCTGCTCAAATAATTGCAAAGAACTTGTTTCAA
 Cys Gln Asn Ile Asn Gln Lys Phe Leu Leu Gln Ile Ile Ala Lys Asn Leu Phe Ser Thr
 CCCCCACTTTGGAATTCAAAAGTTAGGGTAATTACCAAGGTTGGTGTGTCAC
 Pro Leu Leu Glu Tyr Ser Lys Lys Phe Arg Val Ile Thr Arg Phe Gly Val Cys His Phe
 TCTGGGCTGAGAGGGATTAGGTTTCAGAGAAATAATTGTTTACCGGGAGCCGGT
 Trp Ala Glu Arg Asp Phe Arg Phe Gln Arg Asn Lys Leu Cys Phe Thr Gly Ser Arg Cys
 GTTGTCCATGTAGGTTCAAGGGCTTTAGAAATTAGGTGTAATTGTTCCGGCACTTGTG
 Cys Pro Cys Arg Phe Arg Ala Phe Arg Asn Phe Arg Cys Asn Cys Ser Gly Thr Cys Gly
 GTTCTTCAGGTTGGTCTGGCGGTTGGGCCGGGGCGTGTAGGTGAGAAGGG
 Ser Phe Arg Phe Gly Ser Trp Arg Phe Gly Pro Gly Ala Ser Phe Arg Cys Arg Arg Asp
 ATAGATGTAGTTGCTGGGAGCAGGTGTCGTAGGCTGCATTCTGGACTGGTAAAGATT
 Arg Cys Ser Leu Leu Gly Ser Arg Cys Arg Arg Leu His Phe Trp Thr Gly Lys Asp Phe
 TCCAGTTTGAGGAACAAATGGTGTTCACTTGGAGCCAGTGGTGCCTGGCTGTTCA
 Gln Phe Leu Arg Asn Lys Trp Cys Phe Thr Trp Ser Gln Cys Cys Pro Trp Leu Phe Lys
 AGAGTTCTAGAACAGTTAGGTGGGATAGAACATCCAGAACATCGATCACTTGGACTGGGTAG
 Ser Ser Arg Ser Phe Arg Trp Asp Arg Ile Gln Asn Thr Ile Thr Cys Cys Trp Val Gly
 GAATCTGATATCTCAGGCTCATCTAATGTTGAGGGCTTGAGAAAACATCATAAGTTGCA
 Ile
 GTTTGAGGCTGCAGAACATTGGAATCTTCCAGAATTCCCTGAGGCAAAACACCCTTCC
 CTTTGAAAAACCTAG

Figure 5. Clone 3352358-1

CACTTCCCCCTTTGTTAATTAAAATAAGAAGTCGAATGGGAACGAGGTGCCAGCTC
 CCGTGGAGAAAGCTTAAGGACACCACGCCAGTGCTTCCCTGCCTCCCTCGAGATGGAA
 AGAGGAGCTCCTAGCTCACTTAAGCCGGGTAGGGCTGGTTCTCCTTCCGAGCCAAAT
 CCCAGGCGATGGTGAATTATGAACGTGCCACACCATGAAGCTTGTGGCAGGTAACGT
 MetLysLeuLeuTrpGlnValThrVal
 GCACCACACACCTGGAATGCCATCCTGCTCCCGTCTCGTCTACCTCACGGCGAAGTGTG
 HisHisHisThrTrpAsnAlaIleLeuLeuProPheValTyrLeuThrAlaGlnValTrp
 GATTCTGTGAGCCATCGCTGCTGCCCTCAGCCGGCCCCAGAACTGCCCTCCGT
 IleLeuCysAlaAlaIleAlaAlaAlaSerAlaGlyProGlnAsnCysProSerVal
 CTGCTCGTGCAGTAACCAAGTCAGCAAGGTGGTGTGCCACGCCGGGGCTCTCCGAGGT
 CysSerCysSerAsnGlnPheSerLysValValCysThrArgArgGlyLeuSerGluVal
 CCCGCAGGGTATTCCCTCGAACACCCGGTACCTCAACCTCATGGAGAACAAACATCCAGAT
 ProGlnGlyIleProSerAsnThrArgTyrLeuAsnLeuMetGluAsnAsnIleGlnMet
 GATCCAGGGCGACACCTCCGCCACCTCACCACCTGGAGGTCTGCAGTTGGCAGGAA
 IleGlnAlaAspThrPheArgHisLeuHisHisLeuGluValLeuGlnLeuGlyArgAsn
 CTCCATCCGGCAGATTGAGGTGGGGGCCCTCAACGGCCTGCCAGCCTCAGCACCCCTGGA
 SerIleArgGlnIleGluValGlyAlaPheAsnGlyLeuAlaSerLeuSerThrLeuGlu
 GCTGTTCGACAACGGCTGACAGTCATCCCTAGCGGGGCTTGGATAACCTGTCCAAGCT
 LeuPheAspAsnTrpLeuThrValIleProSerGlyAlaPheGluTyrLeuSerLysLeu
 GCAGGGAGCTCTGGCTCGAACAAACCCATCGAAAGCATCCCCTTACGCCCTCAACCG
 ArgGluLeuTrpLeuArgAsnAsnProIleGluSerIleProSerTyrAlaPheAsnArg
 GGTGCCCTCCCTCATGCGCTGGACTTGGGGAGCTCAAGAACAGCTGGAGTATATCTCTGA
 ValProSerLeuMetArgLeuAspLeuGlyGluLeuLysLeuGluTyrIleSerGlu
 GGGAGCTTTGAGGGCTGTTCAACCTCAAGTATCTGAACGGCATGTGCAACATTAA
 GlyAlaPheGluGlyLeuPheAsnLeuLysTyrLeuAsnLeuGlyMetCysAsnIleLys
 AGACATGCCAATCTCACCCCCCTGGTGGGGCTGGAGGAGCTGGAGATGTCAGGGAACCA
 AspMetProAsnLeuThrProLeuValGlyLeuGluGluLeuGluMetSerGlyAsnHis
 CTTCCCTGAGATCAGGCCCTGGCTCCTTCATGGCCTGAGCTCCCTCAAGAACAGCTGGGT
 PheProGluIleArgProGlySerPheHisGlyLeuSerSerLeuLysLeuTrpVal
 CATGAACTCACAGGTCAGCCTGATTGAGCGGAATGCTTGCACGGCTGGCTTCACTTGT
 MetAsnSerGlnValSerLeuIleGluArgAsnAlaPheAspGlyLeuAlaSerLeuVal
 GGAACACTGGCCCACAATAACCTCTTCTTGCCTCATGACCTCTTACCCCGCT
 GluLeuAsnLeuAlaHisAsnAsnLeuSerSerLeuProHisAspLeuPheThrProLeu
 GAGGTACCTGGTGGAGTTGCATCTACACCACAAACCCCTGGAACTGTGATTGTGACATTCT
 ArgTyrLeuValGluLeuHisLeuHisHisAsnProTrpAsnCysAspCysAspIleLeu
 GTGGCTAGCCTGGTGGCTTCGAGAGTATATAACCCACCAATTCCACCTGCTGTGGCCGCTG
 TrpLeuAlaTrpTrpLeuArgGluTyrIleProThrAsnSerThrCysCysGlyArgCys
 TCATGCTCCCATGCACATGCGAGGCCGCTACCTCGTGGAGGTGGACCAGGCCCTTCCA
 HisAlaProMetHisMetArgGlyArgTyrLeuValGluValAspGlnAlaSerPheGln
 GTGCTCTGCCCTTCATCATGGACGACCTCGAGACCTCAACATTCTGAGGGTCGGAT
 CysSerAlaProPheIleMetAspAlaProArgAspLeuAsnIleSerGluGlyArgMet
 GGCAGAACTTAAGTGTGGACTCCCCCTATGTCCTCCGTGAAGTGGTGTGCTGCCCAATGG
 AlaGluLeuLysCysArgThrProProMetSerSerValLysTrpLeuLeuProAsnGly
 GACAGTGCCTAGGCCACGCCCTCCGCCACCCAAGGGATCTCTGCTCTCAACGACGGCACCTT
 ThrValLeuSerHisAlaSerArgHisProArgIleSerValLeuAsnAspGlyThrLeu
 GAACTTTCCACGTGCTGCTTCAGACACTGGGGTGTACACATGCATGGGACCAATGT
 AsnPheSerHisValLeuLeuSerAspThrGlyValTyrThrCysMetGlyThrAsnVal
 TGCAGGCAACTCCAACGCCCTGGCCTACCTCAATGGGAGCAGGCTGAGCTAACACCTC
 AlaGlyAsnSerAsnAlaSerAlaTyrLeuAsnGlySerThrAlaGluLeuAsnThrSer

CAACTACAGCTTCTCACCAAGGAACAGGGGAGACCACGGAGATCTCGCCTGAGGACAC
AsnTyrSerPhePheThrThrGlyThrGlyGluThrThrGluIleSerProGluAspThr
AACCGGAAAGTACAAGCCTGTTCCCTACCACGTCCACTGGTTACCAGGCCGCATATACCAC
ThrArgLysTyrLysProValProThrThrSerThrGlyTyrGlnProAlaTyrThrThr
CTCTACCACGGTGCTCATTCAAGACTACCCGTGTGCCAAGCAGGTGGCAGTACCCGCGAC
SerThrThrValLeuIleGlnThrThrArgValProLysGlnValAlaValProAlaThr
AGACACCACGTACAAGATGCAGACCAGCCTGGATGAAGTCATGAAGAGACCACCAAGATCAT
AspThrThrAspLysMetGlnThrSerLeuAspGluValMetLysThrThrLysIleIle
CATTGGCTGTTGTGGCAGTGACTCTGCTAGCTGCCGCATGTGATTGTCTTCTATAA
IleGlyCysPheValAlaValThrLeuLeuAlaAlaMetLeuIleValPheTyrLys
ACTTCGTAAGCGGCACCAGCAGCGGAGTACAGTCACAGCCGCCGGACTGTTGAGATAAT
LeuArgLysArgHisGlnGlnArgSerThrValThrAlaAlaArgThrValGluIleIle
CCAGGTGGACGAAGACATCCCAGCAGCAACATCCGCAGCAGCAACAGCAGCTCCGTCCGG
GlnValAspGluAspIleProAlaAlaThrSerAlaAlaAlaThrAlaAlaProSerGly
TGTATCAGGTGAAGGGGCAGTAGTGCTGCCACAATTGACCATATTAACATACAACAC
ValSerGlyGluGlyAlaValValLeuProThrIleHisAspHisIleAsnTyrAsnThr
CTACAAACCAGCACATGGGCCACTGGACAGAAAAACAGCCTGGGAACTCTGCACCC
TyrLysProAlaHisGlyAlaHisTrpThrGluAsnSerLeuGlyAsnSerLeuHisPro
CACAGTCACCACTATCTGAACTTATATAATTGACACCATACCAAGGACAAGGTACA
ThrValThrThrIleSerGluProTyrIleIleGlnThrHisThrLysAspLysValGln
GGAAAATCAAATATGACTCCCCCTCCCCAAAAAAACTTATAAAATGCAATAGAATGCACAC
GluThrGlnIleEndLeuProSerProLysLysLeuIleLysCysAsnArgMetHisThr
AAAGACAGCAACTTTGTACAGAGTGGGGAGAGACTTTCTTGATATGCTTATATATT
LysThrAlaThrPheValGlnSerGlyGluArgLeuPheLeuValTyrAlaTyrIleLeu
AAGTCTATGGCTGGTAAAAAAACAGATTATATTAAAGACAAAAAGTC
SerLeuTrpAlaGly

A

Figure 6. Clone 3352358-2

CACCTCCCCCTTTGTTAATTAAAACAAGAAGTCGAATGGAACGAGGTGCCAGCTC
 CCGTGGAGAAAGCTTAAGGACACCACGCCAGTGCTTCCGCCCTCCGAGATGGAA
 AGAGGAGCTCCTAGCTCACTTAAGCCGGGTAGGGCTGGTCTCCTTCCGAGCCAAAT
 CCCAGGCATGGTGAATTATGAACGTGCCACACCCTGAAGCTCTGTGGCAGGTAACGT
 MetLysLeuLeuTrpGlnValThrVal
 GCACCACACACCTGGAATGCCATCCTGCTCCCGTCTACCTCACGGCGCAAGTGTG
 HisHisHisThrTrpAsnAlaIleLeuLeuProPheValTyrLeuThrAlaGlnValTrp
 GATTCTGTGTGCAGCCATCGCTGCCGCCAGCCGGGCCCCAGAACTGCCCTCCG
 IleLeuCysAlaAlaIleAlaAlaAlaSerAlaGlyProGlnAsnCysProSerVal
 CTGCTCGTGCAGTAACCAGTTCAGCAAGGTGGTGTGCACGCCGGGGCCTCTCCGAGGT
 CysSerCysSerAsnGlnPheSerLysValValCysThrArgArgGlyLeuSerGluVal
 CCCCCAGGGTATTCCCTCGAACACCCCGTACCTCACCTCATGGAGGTCCTGCAGTTGGGCAGGAA
 ProGlnGlyIleProSerAsnThrArgTyrLeuAsnLeuMetGluAsnAsnIleGlnMet
 GATCCAGGCCGACACCTCCGCCACCTCACCCACCTGGAGGTCCTGCAGTTGGGCAGGAA
 IleGlnAlaAspThrPheArgHisLeuHisLeuGluValLeuGlnLeuGlyArgAsn
 CTCCATCCGGCAGATTGAGGTGGGGCCCTCAACGGCCTGGCCAGCCTCAGCACCTGG
 SerIleArgGlnIleGluValGlyAlaPheAsnGlyLeuAlaSerLeuSerThrLeuGlu
 GCTGTTCGACAACGGCTGACAGTCATCCCTAGGGGGCCTTGAATACCTGTCCAAGCT
 LeuPheAspAsnTrpLeuThrValIleProSerGlyAlaPheGluTyrLeuSerLysLeu
 GCGGGAGCTCTGGCTTCGCAACAACCCCCTCGAAAGCATCCCTTACGCCCTCAACCG
 ArgGluLeuTrpLeuArgAsnAsnProIleGluSerIleProSerTyrAlaPheAsnArg
 GGTGCCCTCCCTCATGCCCTGGACTTGGGGAGCTCAAGAACGCTGGAGTATATCTCTGA
 ValProSerLeuMetArgLeuAspLeuGlyGluLeuLysLysLeuGluTyrIleSerGlu
 GGGAGCTTTGAGGGGCTGTTCAACCTCAAGTATCTGAACGGCATGTGCAACATTAA
 GlyAlaPheGluGlyLeuPheAsnLeuLysTyrLeuAsnLeuGlyMetCysAsnIleLys
 AGACATGCCAACATCTCACCCCCCTGGTGGGGCTGGAGGAGCTGGAGATGTCAGGGAACCA
 AspMetProAsnLeuThrProLeuValGlyLeuGluGluLeuGluMetSerGlyAsnHis
 CTCCCTGAGATCAGGCCTGGCTCCTCCATGGCCTGAGCTCCCTCAAGAACGCTGGGT
 PheProGluIleArgProGlySerPheHisGlyLeuSerSerLeuLysLysLeuTrpVal
 CATGAACTCACAGGTGAGCTGATTGAGCGGAATGCTTTGACGGGCTGGCTTCACTTGT
 MetAsnSerGlnValSerLeuIleGluArgAsnAlaPheAspGlyLeuAlaSerLeuVal
 GGAACACTAACCTGGCCCACAATAACCTCTCTTGTCCCCATGACCTCTTACCCGCT
 GluLeuAsnLeuAlaHisAsnAsnLeuSerSerLeuProHisAspLeuPheThrProLeu
 GAGGTACCTGGTGGAGTTGCATCTACACCACACCCCTGGAACGTGATTGTGACATTCT
 ArgTyrLeuValGluLeuHisLeuHisAsnProTrpAsnCysAspCysAspIleLeu
 GTGGCTAGCCTGGTGGCTTCGAGAGTATATACCCACCAATTCCACCTGCTGTGGCCGCTG
 TrpLeuAlaTrpTrpLeuArgGluTyrIleProThrAsnSerThrCysCysGlyArgCys
 TCATGCTCCCATGCACATGCGAGGCCGCTACCTCGTGGAGGTGGACCAGGCCCTTCCA
 HisAlaProMetHisMetArgGlyArgTyrLeuValGluValAspGlnAlaSerPheGln
 GTGCTCTGCCCTTCATCATGGACGCCACCTCGAGACCTCAACATTCTGAGGGTCGGAT
 CysSerAlaProPheIleMetAspAlaProArgAspLeuAsnIleSerGluGlyArgMet
 GGCAGAACTTAAGTGTGCGACTCCCCCTATGTCCTCCGTGAAGTGGTTGCTGCCCAATGG
 AlaGluLeuLysCysArgThrProProMetSerSerValLysTrpLeuLeuProAsnGly
 GACAGTGTGCTAGCCACGCCCTCCGCCACCAAGGATCTGTCTCAACGACGGCACCTT
 ThrValLeuSerHisAlaSerArgHisProArgIleSerValLeuAsnAspGlyThrLeu
 GAACTTTCCACGTGCTTCAAGACACTGGGGTGTACACATGCATGGGACCAATGTT
 AsnPheSerHisValLeuLeuSerAspThrGlyValTyrThrCysMetGlyThrAsnVal
 TGAGGCAACTCCAACGCCCTGGCCTACCTCAATGGAGCACGGCTGAGCTTAACACCTC
 AlaGlyAsnSerAsnAlaSerAlaTyrLeuAsnGlySerThrAlaGluLeuAsnThrSer

CAACTACAGCTTCTTACCCACAGGAACAGGGGAGACCACGGAGATCTGCCCTGAGGACAC
AsnTyrSerPhePheThrThrGlyThrGlyGluThrThrGluileSerProGluAspThr
AACCGCAAAGTACAAGCCTGTTCTACCACGTCCACTGGTTACCAGCCGGCATATACCAC
ThrArgLysTyrLysProValProThrThrSerThrGlyTyrGlnProAlaTyrThrThr
CTCTACCACGGTGCCTATTCACTACCCGTGTGCCAAGCAGGTGGCAGTACCCGCGAC
SerThrThrValLeuIleGlnThrThrArgValProLysGlnValAlaValProAlaThr
AGACACCACGTACAAGATGCAGACCAGCCTGGATGAAGTCATGAAGACCACCAAGATCAT
AspThrThrAspLysMetGlnThrSerLeuAspGluValMetLysThrThrLysIleIle
CATTGGCTGCTTGTGGCAGTGACTCTGCTAGCTGCCCATGTTGATTGTCTTCTATAA
IleGlyCysPheValAlaValThrLeuLeuAlaAlaMetLeuIleValPheTyrLys
ACTTCGTAAGCGGCACCAGCAGCGGAGTACAGTCACAGCCGCCAACACTGGAGAGAAA
LeuArgLysArgHisGlnGlnArgSerThrValThrAlaAlaProThrLeuGluArgLys
ACACAGGGACAAAAACACACCACAAACAAAAACCCCCACAACAAAAACAGCCCCCCC
HisArgAspLysAsnThrProGlnGlnLysHisProGlnGlnLysGlnGlnProProPro
GGTATAACAGGAAAGGGCACAAATAGCCCCCACAAAACACAACATAAAAAACAAAAC
Val
ACACACAAACCCAGCACATGGGCCACTGGACAGAAAACAGCCTGGGAACCTGTGCAC
CCACACAGTCACCACTATCTGAACCTTATATAATTCAAGCCCATACCAAGGACAAGGTA
CAGGAAACTCAAATATGACTCCCTCCCCAAAAAACTTATAAAATGCAATAGAATGCAC
ACAAAGACAGCAACTTTGTACAGAGTGGGAGAGACTTTCTGTATATGCTTATATA
TTAAGTCTATGGGCTGGTTAAAAAAACAGATTATTTAAAGACAAAAAGTCA
AAACAAAAATATTTCTAATTGTAAGTTCTATTAAAGGGGTGGGGGGAAATCTTGGG
AACGTTGTGGGTACAAGCCACAAGTTAACCTGCTATGCTGCCAGAAGGGATTCTGGTA
TAAGGTTGAAATTGCTGAGATAAAACTAAACAAACATCCTTAAAGAGGTAGG
GTGTGGGCTGCTGAAGGGCAAGAGGGATAGACTGAATCTGTCATTTAGAAGATGCTT
CATAGGACACAGGACTATCCATTCTA

Figure 7**7A. Clone 3884846**

GGCCTTGTGCCGTCCGGCTNCCTGACATGCAGATTCCACCCAGAAGACAGAGAAGG
 AGCCAGTGGTCATGGAATGGCTGGGTCAAAGACTGGGTGCCTGGAGCTGAGGCAGCC
 ACCGTTCAGCCTGGCCAGCCTCTGGACCCCGAGGTTGGACCCACTGTGACACACCTA
 CCATGCGGACACTCTCAACCTCTCTGGCTTGCCCTGGCTGCAGCCCTGTTCACACTA
 CCCTGTCAGATGCAAAAGCCGCTCAAAGACGCTGGAGAAGAGTCAGT
 TTTCAGATAAGCCGGTCAAGACGGGGTTGGTGGACGGACTCAAAGCTGAGAGTG
 TGGTTCTTGAGCATCGCAGCTACTGCTCGGCAAAGGCCGGACAGACACTTGTGCTGGGG
 ATGTAATGGCTATGTCACTCCACCAGTGGAACAGCCATGGCTACGATGTCACCAAGGTC
 MetTyrTrpAlaMetSerLeuHisGlnTrpAsnSerHisGlyTyrAspValThrLysVal

 TTGGGAGCAAGTTCACACAGATCTCACCCGTCTGGCTGCAGCTGAAGAGACGTGGCCGT
 PheGlySerLysPheThrGlnIleSerProValTrpLeuGlnLeuLysArgArgGlyArg

 GAGATTTGAGGTACGGGCTCCACGACGTGGACCAAGGGTGGATGCGAGCTGTCAGG
 GluMetPheGluValThrGlyLeuHisAspValAspGlnGlyTrpMetArgAlaValArg

 AAGCATGCCAAGGGCCTGCACATAGTCGGCTCGGCTCTGGTGGAGGACTGGACTTACGAT
 LysHisAlaLysGlyLeuHisIleValProArgLeuLeuPheGluAspTrpThrTyrAsp

 GATTCCGGAACGTCTTAGACAGTGAGGATGAGATAGAGGAGCTGAGCAAGACCGTGGTC
 AspPheArgAsnValLeuAspSerGluAspGluIleGluGluLeuSerLysThrValVal

 CAGGTGGCAAAGAACGACGATTCGATGGCTTCTGGTGGAGGCTGGAACCCAGCTGCTA
 GlnValAlaLysAsnGlnHisPheAspGlyPheValValGluValTrpAsnGlnLeuLeu

 AGCCAGAACGCGTGGGCTCATCCACATGCTACCCACTGGCCGAGGCTGCACCAAG
 SerGlnLysArgValGlyLeuIleHisMetLeuThrHisLeuAlaGluAlaLeuHisGln

 GCCCGCTGCTGGCCCTCTGGTCATCCGCCTGCATCACCCGGGACCGAGCTG
 AlaArgLeuAlaLeuLeuValIleProProAlaIleThrProGlyThrAspGlnLeu

 GGCATGTTCACCCACAAGGAGTTGAGCAGCTGGCCCCGTGCTGGATGGTTTCAGCCTC
 GlyMetPheThrHisLysGluPheGluGlnLeuAlaProValLeuAspGlyPheSerLeu

 ATGACCTACGACTACTCTACAGCGCATCAGCCTGGCCCTAACGACCCCTGTCCTGGTT
 MetThrTyrAspTyrSerThrAlaHisGlnProGlyProAsnAlaProLeuSerTrpVal

 CGAGCCTCGTCCAGGTCTGGACCCGAAGTCCAAGTGGCGAAGCAAAATCCTCCTGGGG
 ArgAlaCysValGlnValLeuAspProLysSerTrpArgSerLysIleLeuGly

 CTCAACTTCTATGGTATGGACTACGCGACCTCAAGGATGCCGTGAGCCTGTTGTCGGG
 LeuAsnPheTyrGlyMetAspTyrAlaThrSerLysAspAlaArgGluProValValGly

 GCCAGGTACATGCAGACACTGAAAGTCTGCATTGACTCTTAGCAAACCTGGAAAATTG
 AlaArgTyrMetGlnThrLeuLysSerAlaPheValLeuAlaAsnLeuGluAsnLeu

 AGGCGAAATTCTCAAATAAAAAAAAAAAAAAATTTTCTGTTCTTCTTTCT
 ArgArgAsnSerSerAsnLysLysLysLysLysAsnPhePheCysPhePheSerSer

 GTCTTCTCGTTGGAGACCACAAACACTAGATCCATTGAATTGTCCCACAGCTCACGAA
 ValPheSerPheGlyAspHisLysHis

 TACACCTTTACCTTTGGA

7B. Clone 3884846-1

1 GCCTCCCTGACATGCAGCCCTGGACCCCGAGGTGGACCTAC
 AlaSerLeuThrCysSerProLeuAspProGluValGlyProTyr
 46 TGTGACACACCTACCAGCGGACACTCTTCACCTCCTCTGGCTT
 CysAspThrProThrMetArgThrLeuPheAsnLeuLeuTrpLeu
 91 GCCCTGGCCTGCAGCCCTGTTCACACTACCCTGTCAAAGTCAGAT
 AlaLeuAlaCysSerProValHisThrThrLeuSerLysSerAsp
 136 GCCAAAAAAGCCGCCTCAAAGACGCTGCTGGAGAAAGAGTCAGTTT
 AlaLysLysAlaAlaSerLysThrLeuLeuGluLysSerGlnPhe
 181 TCAGATAAGCCGGTGCAGACCGGGTTGGTGGTGACGGACCTC
 SerAspLysProValGlnAspArgGlyLeuValValThrAspLeu
 226 AAAGCTGAGAGTGTGGTTCTTGAGCATCGCAGCTACTGCTCGGCA
 LysAlaGluSerValValLeuGluHisArgSerTyrCysSerAla
 271 AAGGCCCGGACAGACACTTGCTGGGATGTACTGGGCTATGTC
 LysAlaArgAspArgHisPheAlaGlyAspValLeuGlyTyrVal
 316 ACTCCATGGAACAGCCATGGCTACGATGTCACCAAGGTCTTG
 ThrProTrpAsnSerHisGlyTyrAspValThrLysValPheGly
 361 AGCAAGTTCACACAGATCTCACCCGCTGGCTGCAGCTGAAGAGA
 SerLysPheThrGlnIleSerProValTrpLeuGlnLeuLysArg
 406 CGTGGCCGTGAGATGTTGAGGTACGGCTACGGGCCTCCACGACGTGGAC
 ArgGlyArgGluMetPheGluValThrGlyLeuHisAspValAsp
 451 CAAGGGTGGATGCGAGCTGTCAGGAAGCATGCCAAGGGCCTGCAC
 GlnGlyTrpMetArgAlaValArgLysHisAlaLysGlyLeuHis
 496 ATAGTGCCTCGGCTCCTGTTGAGGACTGGACTTACGATGATTTC
 IleValProArgLeuLeuPheGluAspTrpThrTyrAspAspPhe
 541 CGGAACGTCTTAGACAGTGAGGATGAGATAGAGGAGCTGAGCAAG
 ArgAsnValLeuAspSerGluAspGluIleGluGluLeuSerLys
 586 ACCGTGGTCCAGGTGGAAAGAACCAACGACATTGATGGCTTCGTG
 ThrValValGlnValAlaLysAsnGlnHisPheAspGlyPheVal
 631 GTGGAGGTCTGGAACCAAGCTGCTAACGCCAGAGCGCGTGGGCCTC
 ValGluValTrpAsnGlnLeuLeuSerGlnLysArgValGlyLeu
 676 ATCCACATGCTACCCACTGGCCGAGGCTCTGCACCAAGGCCGG
 IleHisMetLeuThrHisLeuAlaGluAlaLeuHisGlnAlaArg
 721 CTGCTGGCCCTCCTGGTCATCCCGCTGCCCCATCACCCCGGGACC
 LeuLeuAlaLeuLeuValIleProProAlaIleThrProGlyThr
 766 GACCAGCTGGGCATGTTCACGCACAAGGAGTTGAGCAGCTGGCC
 AspGlnLeuGlyMetPheThrHisLysGluPheGluGlnLeuAla
 811 CCCGTGCTGGATGGTTCAAGCCTCATGACCTACGACTACGCAACA
 ProValLeuAspGlyPheSerLeuMetThrTyrAspTyrAlaThr
 856 CTGTCCTGGGTTCGAGCCTGCGTCCAGGTGCTGGATCCCTGGGC
 LeuSerTrpValArgAlaCysValGlnValLeuAspProTrpGly

901 TCAACTTCTATGGTATGGACTACCGCACCTCCAAGGATGCCCGTG
SerThrSerMetValTrpThrThrArgProProArgMetProVal

946 AGCCTGTTGTGGGGCCAGGTACATCCAGACACTGAAGGACCACA
SerLeuLeuSerGlyProGlyThrSerArgHis

991 GGCCCCGGATGGTGTGGGACGGCCAGGCCTCAGAGCACTTCCTTCG

1036 AGTACAAGAAGAGCCGCAGTGGGAGGCACGTCGTCTTCTACCCAA

1081 CCCTGAAGTCCCTGCAGGTGCGGCTGGAGCTGGCCGGAGCTGG

1126 GCGTTGGGTCTCNATNTGGGAGCTGGCCAGGGCTGGACTACT

1171 TNTACGACCTGCTTAGGTGGCATTCGGCCTCCGGTGGACG

1216 TGTTCTTTCTAAGCCATGGAGTGAGTGAGCAGGTGTGAAATACA

1261 GGCTCCACTCCGTTACAAAAAAA

7C. Clon 3884846-2

1 GCCTCCCTGACATGCAGCCCTCTGGACCCCGAGGTTGGACCCCTAC
 46 TGTGACACACCTTACCATGCGGACACTCTTCACCTCCCTCTGGCTT
 91 GCCCTGGCCTGCAGCCCTGTTACACTACCCTGTCAAAGTCAGAT
 136 GCAAAAAAGCCGCCCTCAAAGACGCTGCTGGAGAAGAGTCAGTT
 181 TCAGATAAGCCGGTGCAAGACCGGGGTTTGGTGGTGACGGACCTC
 226 AAAGCTGAGAGTGTGGTTCTTGAGCATCGCAGCTACTGCTCGGCA

 271 AAGGCCCGGGACAGACACTTGTGGATGTACTGGCTATGTC
 MetTyrTrpAlaMetSe

 316 ACTCCACCAGTGGAACAGCCATGGCTACGATGTCACCAAGGTCTT
 rLeuHisGlnTrpAsnSerHisGlyTyrAspValThrLysValPh

 361 TGGGAGCAAGTTCACACAGATCTCACCCGTCTGGCTGCAGCTGAA
 eGlySerLysPheThrGlnIleSerProValTrpLeuGlnLeuLy

 406 GAGACGTGGCCGTGAGATGTTGAGGTACGGGCCTCCACGAGT
 sArgArgGlyArgGluMetPheGluValThrGlyLeuHisAspVa

 451 GGACCAAGGGTGGATGCGAGCTGTCAGGAAGCATGCCAAGGGCT
 lAspGlnGlyTrpMetArgAlaValArgLysHisAlaLysGlyLe

 496 GCACATAGTGCCTCGGCTCTGTTGAGGACTGGACTTACGATGA
 uHisIleValProArgLeuLeuPheGluAspTrpThrTyrAspAs

 541 TTTCCGGAACGTCTTAGACAGTGAGGATGAGATAGAGGAGCTGAG
 pPheArgAsnValLeuAspSerGluAspGluIleGluGluLeuSe

 586 CAAGACCGTGGTCCAGGTGGAAAGAACCGACATTGATGGCTT
 rLysThrValValGlnValAlaLysAsnGlnHisPheAspGlyPh

 631 CGTGGTGGAGGTCTGGAACCAGCTGCTAACGCCAGAACGCGGTGG
 eValValGluValTrpAsnGlnLeuLeuSerGlnLysArgValGl

 676 CCTCATCCACATGCTCACCCACTTGGCCGAGGCTCTGCACCAGGC
 yLeuIleHisMetLeuThrHisLeuAlaGluAlaLeuHisGlnAl

 721 CCGGCTGCTGGCCCTCCTGGTCATCCCCGCTGCCATCACCCCCGG
 aArgLeuLeuAlaLeuLeuValIleProProAlaIleThrProGl

 766 GACCGACCAGCTGGGCATGTTCACGCACAAGGAGTTGAGCAGCT
 yThrAspGlnLeuGlyMetPheThrHisLysGluPheGluGlnLe

 811 GGCCTCCCGTGGATGGTTGACGCTCATGACCTACGACTACGC
 uAlaProValLeuAspGlyPheSerLeuMetThrTyrAspTyrAl

 856 AACACTGTCTGGGTCAGCCTGCGTCCAGGTCTGGATCCCTG
 aThrLeuSerTrpValArgAlaCysValGlnValLeuAspProTr

 901 GGGCTCAACTCTATGGTATGGACTACGCGACCTCCAAGGATGCC
 pGlySerThrSerMetValTrpThrThrArgProProArgMetPr

 946 CGTGAGCCTGTTGTCGGGCCAGGTACATCCAGACACTGAAGGAC
 oValSerLeuLeuSerGlyProGlyThrSerArgHis

991 CACAGGCCCGGATGGTGTGGGACGGCAGGCCTCAGAGCACTTC
1036 TTGAGTACAAGAAGAGCCGAGTGGGAGGCACGTCTGCTTCTAC
1081 CCAACCTGAAGTCCCTGCAGGTGCAGGCTGGAGCTGGCCAGGGCTGGAC
1126 CTGGCGTTGGGTCTCNATNTGGGAGCTGGCCAGGGCTGGAC
1171 TACTTNTACGACCTGCTTAGGTGGGATTGCGGCCTCCGCGGTG
1216 GACGTGTTCTTTCTAACGGCATGGAGTGAGTGAGCAGGTGTGAAA
1261 TACAGGCCTCCACTCCGTTACAAAAAAAAAA

Figure 8.**8A. Clone 3911675**

```

ACCGGTGCAGGTGGCGGAACTTGCTCTAACCTCCTCGGCCAGCCGGCCGCCGCCGC
TGCGCCGCCGCGCGGGATTCTGCTTCAGAAAGATGCACTATTATAGATACTCTAACG
MetHisTyrArgTyrSerAsnAla

CCAAGGTCAGCTGCTGGTACAAGTACCTCCTTTCAGCTACAACATCATCTCTGGTTGG
LysValSerCysTrpTyrLysTyrLeuLeuPheSerTyrAsnIleIlePheTrpLeuAla

CTGGAGTTGTCTTCCTGGAGTCGGGTTGTGGCATGGAGCGAAAAGGGTGTGCTGTCCG
GlyValValPheLeuGlyValGlyLeuTrpAlaTrpSerGluLysGlyValLeuSerAsp

ACCTCACCAAAGTGACCCGGATGCATGGAATCGACCCTGCGGTGCTGGTCCTGATGGTGG
LeuThrLysValThrArgMetHisGlyIleAspProAlaValLeuValLeuMetValGly

GCGCGGTGATGTTCACCCGGGGTCGCCGGCGCGTGGGGCGCGCAGGGAGAATATCT
AlaValMetPheThrLeuGlyPheAlaGlyArgValGlyAlaArgArgGluAsnIleCys

GCTTGCTCAACTTTTCTGTGGCACCATCGTGCATCTTCTTCCTGGAGCTGGCTGTGG
LeuLeuAsnPhePheCysGlyThrIleValLeuIlePhePheLeuGluLeuAlaValAla

CCGTGCTGGCCTTCCTGTTCCAGGACTGGGTGAGGGACCGGTTGGGAGTTCTCGAGA
ValLeuAlaPheLeuPheGlnAspTrpValArgAspArgPheArgGluPhePheGluSer

GCAACATCAAGTCCTACCGGGACGATATCGATCTGCAAAACCTCATCGACTCCCTCAGA
AsnIleLysSerTyrArgAspAspIleAspLeuGlnAsnLeuIleAspSerLeuGlnLys

AAGCTAACCAAGTAGCTGTGGCGCATATGCCCTGAAGACTGGGACCTAACGTCTACTTCA
AlaAsnGlnCysCysGlyAlaTyrGlyProGluAspTrpAspLeuAsnValTyrPheAsn

ATTGCAGCGGTGCCAGCTACAGCCGAGAGAAAGTGCAGGGTCCCCCTCTCCTGCTGCGTGC
CysSerGlyAlaSerTyrSerArgGluLysCysGlyValProPheSerCysCysValPro

CAGATCCTGCGAAAAAGTTGTGAACACACAGTGTGGATATGATGTCAGGATTCAAGCTGA
AspProAlaGlnLysValValAsnThrGlnCysGlyTyrAspValArgIleGlnLeuLys

AGAGCAAGTGGGATGAGTCATCTCACGAAAGGCTGCATCCAGGCCTGGAAAGCTGGC
SerLysTrpAspGluSerIlePheThrLysGlyCysIleGlnAlaLeuGluSerTrpLeu

TCCCGCGGAACATTACATTGTGGCTGGCGTCTCATGCCATCTCGCTGTTGCAGATAT
ProArgAsnIleTyrIleValAlaGlyValPheIleAlaIleSerLeuLeuGlnIlePhe

TTGGCATCTCTGGCAAGGACGCTGATCTCAGACATCGAGGCACTGAAGACCGGCCATC
GlyIlePheLeuAlaArgThrLeuIleSerAspIleGluAlaValLysThrGlyHisHis

ACTCTGAGGGAGCAGAGTTGAGGGAGCCGAGCTGAGCCACGCTGGGAGGCCAGAGCCTT
Phe

CTCTGCCATCACGCCCTACGTCCAGAGGGAGAGGGAGCCGACACCCCCAGAGCCAGTGC
ATCTTAAGCATCACGCGTGAAGTGCACCTCTGTGTTCTGCTGCTGGCTGAAGACCAAG
GGTCCCCCTTGATACTGCCAAACTTGACTGCATCCCTCTGGAGTCTACCCAGAGAC
AGAGAATGTGCTTTATGTGGGAGTGGTGAATCTGAAAGACAGAGAGGGCTCTGTGGCT
GCCAGGAGGGCTTGACTCAGACCCCTGCAGCTCAAGCATGTCAGGACACCCCTGGTC
CCCTCTCCACTGGCAGACATCTGCTTGGGTGATCCACATCTGTGGGTGGGCCGTG
GGTAGAGGGACCCACAGGGCTGGACAGGGCATCTCTCCATCAAGCAAAGCAGCATGGG
GGCCTGCCGTAACGGGAGGGAGCTGGCCCCGCTGGGCCCTCTCCGA

```

8B. Clone 3911675-2

1 CCGCGTGTGGTCCTGATGGTGGGCGCGGTGATGTTACCCGGGG
 ArgValLeuValLeuMetValGlyAlaValMetPheThrArgGl
 46 TTCGCCGGCCGCGTGGGGCGCCAGGGAGAATATCTGCTTGCT
 ySerProAlaAlaTrpGlyArgAlaArgGluAsnIleCysLeuLe
 91 CAACCTTTCTGTGGCACCATCGTGCATCTTCTCCTGGAGCT
 uAsnPhePheCysGlyThrIleValLeuIlePhePheLeuGluLe
 136 GGCTGTGGCCGTGCTGGCCTCCTGTTCCAGGACTGGGTGAGGG
 uAlaValAlaValLeuAlaPheLeuPheGlnAspTrpValArgAs
 181 CCGGTTCCGGGAGTTCTCGAGAGCAACATCAAAGTCCCTACCGGG
 pArgPheArgGluPhePheGluSerAsnIleLysSerTyrArgAs
 226 CGATATCGATCTGAAAACCTCATCGACTCCCTTCAGAAAGCTAA
 pAspIleAspLeuGlnAsnLeuIleAspSerLeuGlnLysAlaAs
 271 CCAGTGCTGTGGCGCATATGCCCTGAAGACTGGGACCTAACGT
 nGlnCysCysGlyAlaTyrGlyProGluAspTrpAspLeuAsnVa
 316 CTACTTCAATTGCAGCGGTGCCAGCTACAGCCGAGAGAAGTGC
 GlyIleTyrPheAsnCysSerGlyAlaSerTyrSerArgGluLysCysG
 361 GGTCCCCTTCCTGCTGCGTGCAGATCCTGCGAAAAAGTTGT
 yValProPheSerCysCysValProAspProAlaGlnLysValVa
 406 GAACACACAGTGTGGATATGATGTCAGGATTCAAGCTGAAGAGCAA
 lAsnThrGlnCysGlyTyrAspValArgIleGlnLeuLysSerLy
 451 GTGGGATGAGTCCATCTTCACGAAAGGCTGCATCCAGGCGCTGG
 AAspTrpAspGluSerIlePheThrLysGlyCysIleGlnAlaLeuG
 496 AAGCTGGCTCCCGGAAACATTACATTGTGGCTGGCGTCTTCAT
 uSerTrpLeuProArgAsnIleTyrIleValAlaGlyValPheIl
 541 CGCCATCTCGCTGTCAGATATTGGCATCTCCTGGCAAGGAC
 eAlaIleSerLeuGlnIlePheGlyIlePheLeuAlaArgTh
 586 GCTGATCTCAGACATCGAGGGCAGTGAAGGCCGGCCATCACTTCTG
 rLeuIleSerAspIleGluAlaValLysAlaGlyHisHisPhe
 631 AGGAGCAGAGTTGAGGGAGCCGAGCTGAGCCACGCTGGGAGGGCCA
 676 GAGCCTTCTGCCATCAGCCCTACGTCAGAGGGAGAGGAGGCC
 721 GACACCCCCAGAGCCAGTCCCCATCTTAAGCATCAGCGTGACGT
 766 GACCTCTCTGTTCTGCTTGCTGGTGAAGACCAAGGGTCCCC
 811 CTTGTT

Figure 9.**9A. Clone 4004056**

```

AACGGCGCAGGTCCCAGCAGCTGGGTTCCCCCTCAGCCCGTGAGCAGCCATGTCCAACC
MetSerAsnPro

CCAGCGCCCCACCACCATATGAAGACCGCAACCCCTGTACCCAGGCCCTGTGCCCCCTG
SerAlaProProProTyrGluAspArgAsnProLeuTyrProGlyProLeuProProGly

GGGGCTATGGGCAGCCATCTGCTGCCAGGAGGGTATCCTGCCACCCCTGGCTACCCGC
GlyTyrGlyGlnProSerValLeuProGlyGlyTyrProAlaTyrProGlyTyrProGln

AGCCTGGCTACGGTCACCCGTGGCTACCCACAGCCCATGCCACCCACCCACCCGATGC
ProGlyTyrGlyHisProAlaGlyTyrProGlnProMetProProThrHisProMetPro

CCATGAACTACGGCCACGGCCATGGCTATGATGGGAGGGAGAGAGCGGTGAGTGATAGCT
MetAsnTyrGlyProGlyHisGlyTyrAspGlyGluGluArgAlaValSerAspSerPhe

TCGGGCCTGGAGAATGGGATGACCGGAAAGTGCACACACTTTATCCGAAAGGTTACT
GlyProGlyGluTrpAspAspArgLysValArgHisThrPheIleArgLysValTyrSer

CCATCATCTCGGGCAGCTGCTCATCACTGGGCCATCATTGCTATCTCACCTTGGGG
IleIleSerGlyGlnLeuLeuIleThrGlyAlaIleIleAlaIlePheThrPheGlyGlu

AACCTGTCAGCGCCTTGGCAGGAGAAATGTGGCTGTCTACTACGTGTCCTATGCTGTCT
ProValSerAlaPheGlyArgArgAsnValAlaValTyrTyrValSerTyrAlaValPhe

TCAGTGTACCTACCTGATCCTGCTGCCAGGGACCCAGACGCCGTTCCATGGGA
SerValThrTyrLeuIleLeuAlaCysCysGlnGlyProArgArgPheProTrpAsn

ACATCATTCTGCTGACCCCTTTTACTTTGCCATGGGCTCATGACGGGCACCATTTCCA
IleIleLeuLeuThrLeuPheThrPheAlaMetGlyPheMetThrGlyThrIleSerSer

GTATGTACCAAACCAAAGCCGTACATGCAATGATCATCACTGCGGTGGTATCCATT
MetTyrGlnThrLysAlaValIleIleAlaMetIleIleThrAlaValValSerIleSer

CAGTCACCACCTCTGCTTCAGACCAAGGTGGACTTCACCTCGTCACAGGCCTCTTCT
ValThrIlePheCysPheGlnThrLysValAspPheThrSerCysThrGlyLeuPheCys

GTGTCCTGGATTGTGCTCTGGTACTGGGATTGTCACTAGCATTGTGCTCTACTTCC
ValLeuGlyIleValLeuLeuValThrGlyIleValThrSerIleValLeuTyrPheGln

AATACGTTACTGGCTCCACATGCTCTATGCTGCTGGGGCCATTGTTTACCCCTGT
TyrValTyrTrpLeuHisMetLeuTyrAlaAlaLeuGlyAlaIleCysPheThrLeuPhe

TCCTGGCTTACGACACACAGCTGGCCTGGGAACCGGAAGCACACCACAGCCCCGAGG
LeuAlaTyrAspThrGlnLeuValLeuGlyAsnArgLysHisThrIleSerProGluAsp

ACTACATCACTGGCGCCCTGCAGATTACACAGACATCATCTACATCTTCACCTTGTC
TyrIleThrGlyAlaLeuGlnIleTyrThrAspIleIleTyrIlePheThrPheValLeu

TGCAGCTGATGGGGATCGCAATTAAAGGAGCAAGCCCCATTTCACCGATCTGGGCT
GlnLeuMetGlyAspArgAsn

```

CTCCCTTCCAAGCTAGAGGGCTGGGCCCCATGACTGTGGCTGGGCTTTAGGCCCTTTC
CTTCCCCTTGAGTAACATGCCAGTTCTTCTGTCTGGAGACAGGTGGCCTCTCTGG
CTATGGATGTGGGTACTTGGTGGGACGGAGGAGCTAGGGACTAACTGTTGCTCTTGG
TGGGCTTGGCAGGGACTAGGCTGAAGATGTCTTCTCCCCGCCACCTACTGTATGACAC
CACATTCTTCTAACAGCTGGGTTGTGAGGAATATGAAAAGAGCCTATTGATAGCTAG
AAGGAATATGAAAGGTAGAAGTGACTTCAAGGTACGAGGTTCCCTCCACCTCTGTC
ACAGGCTTCTTGACTACGTAGTTGGAGCTATTCTCCCCCAGCAAAGCCAGAGAGCTTT
GTCCCCGGCCTCTGGACACATAGGCCATTATCCTGATTCTTGGCTTGGCATCTTTT
AGCTCAGGAAGGTAGAAGAGATCTGTGCCCATGGGTCTCCTTGCTTCAATCCCTCTTGT
TTCAGTGACATATGTTATTGTTATCTGGTTAGGGATGGGGACAGATAATAGAACGAGC
AAAGTAACCTATACAGGCCAGCATGGAACAGCATCTCCCTGGGCTTGCCTGGCTTGT
GACGCTATAAGACAGAGCAGGCCACATGTGGCCATCTGCTCCCCATTCTTGAAGCTGCT
GGGGCCTCTTGCAAGGCTTCTGGATCC

9B. Clone 4004056-2

1 CGCTCCGTCTGGAACGGCGCAGGTCCCAGCAGCTGGGTTCCCC
 46 TCAGCCCGTGAGCAGCCATGTCCAACCCAGCGCCCCACCACCAT
 MetSerAsnProSerAlaProProProt
 91 ATGAAGACCGCAACCCCTGTACCCAGGCCCTCTGCCCTGGGG
 yrGluAspArgAsnProLeuTyrProGlyProLeuProProGlyG
 136 GCTATGGGCAGCCATCTGTCCTGCCAGGGAGGTATCCTGCCTACC
 lyTyrGlyGlnProSerValLeuProGlyGlyTyrProAlaTyrP
 181 CTGGCTACCCGCAGCCTGGCTACGGTCACCCCTGCTGGCTACCCAC
 roGlyTyrProGlnProGlyTyrGlyHisProAlaGlyTyrProG
 226 AGCCCATGCCCCCAACCCACCGATGCCATGAACCTACGCCAG
 lnProMetProProThrHisProMetProMetAsnTyrGlyProG
 271 GCCATGGCTATGATGGGGAGGAGAGAGCGGTGAGTGATAGCTTCG
 lyHisGlyTyrAspGlyGluGluArgAlaValSerAspSerPheG
 316 GGCCTGGAGAGTGGGATGACCGGAAAGTGCACACACTTTATCC
 lyProGlyGluTrpAspAspArgLysValArgHisThrPheIleA
 361 GAAAGGTTTACTCCATCATCTCCGTGAGCTGCTCATCACTGTGG
 rgLysValTyrSerIleIleSerValGlnLeuLeuIleThrValA
 406 CCATCATTGCTATCTTCACCTTGTGGAACCTGTCAGCGCCTTG
 laIleIleAlaIlePheThrPheValGluProValSerAlaPheV
 451 TGAGGGAGAACATGTGGCTGTCTACTACCGTGCCTATGCTGTCTTCG
 alArgArgAsnValAlaValTyrTyrValSerTyrAlaValPheV
 496 TTGTCACCTACCTGATCCTGCCGTGCTGCCAGGGACCCAGACGCC
 alValThrTyrLeuIleLeuAlaCysCysGlnGlyProArgArgA
 541 GTTTCCCATGGAACATCATTCTGCTGACCCCTTTACTTTGCA
 rgPheProTrpAsnIleIleLeuLeuThrLeuPheThrPheAlaM
 586 TGGGCTTCATGACGGGCACCATTCCAGTATGTACCAAACCAAAG
 etGlyPheMetThrGlyThrIleSerSerMetTyrGlnThrLysA
 631 CCGTCATCATTGCAATGATCATCACTGCGGTGGTATCCATTTCAG
 laValIleIleAlaMetIleIleThrAlaValValSerIleSerV
 676 TCACCATCTCTGCTTCACTGACCAAGGTGAGGGCATGGAGGGCC
 alThrIlePheCysPheGlnThrLysValArgAlaTrpArgAlaL
 721 TTCCCTGGCCCCCGACTCCCCTTCTTATCAGGCCGGACCCG
 euProTrpProProAspSerProPheLeuSerGlyProAspProG
 766 GTACACTAGGGATGTTCCCTAGAGACCTGATCCCCCTCTCCTCAT
 lyThrLeuGlyMetPheProArgAspLeuIleProPheSerSers
 811 CCGCACCTACAAAATGTGTCTGTTCTGTCCTAGAATGTTGT
 erAlaProThrLysLeuCysProValSerValLeuArgMetLeuT
 856 GGACATTCCCATACCCCTAGGAGGCAGCACTGGGACTCCCTGGC
 rpThrPheProTyrProLeuGlyGlySerThrGlyThrProTrpG

901 AGGGCCAGTCTGACTGGGCTGGTTGTACAGCCATCTGACAGGTG
1nGlyGlnSerAspTrpAlaGlyCysHisSerHisLeuThrGlyA

946 CCTCTTCCTGCTTCCCTGGCAGGTGGACTTCACCTCGTCACAGG
laSerPheLeuLeuProGlyArgTrpThrSerProArgAlaGlnA

991 CCTCTTCTGTGTCCTGGGAATTGTGCTCCTGGTACTGGGATTGT
laSerSerValSerTrpGluLeuCysSerTrp

1036 CACTAGCATTGTGCTCTTAGCATTTGCTCTACTTCCAATACGTT

1081 TACTGGCTCCACATGCTCTATGCTGCTCTGGGGCCATTGTTTC

1126 ACCCTGTTCTGGCTTACGACACACAGCTGGCTCTGGGAACCGG

1171 AAGCACACCATCAGCCCCAGGACTACATCACTGGCCCTGCAG

1216 ATTTACACAGACATCATCTACATCTTACACCTTGCTGCTGCAGCTG

1261 ATGGGGGATCGCAATTAAAGGAGCAAGCCCCCATTTCACCCGATC

1306 CTGGGCTCTCCCTTCAAGCTAGAGGGCTGGGCTCAATGACTGTG

1351 GTCTGGGCTTTAGGCCCTTCTCCCTTGAGTAACATGCCA

1396 GTTTCCCTTCTGTCTGGAGACAGGTGGCCTCTCTGGCTATGGAT

1441 GTGTGGGTACTTGGTGGGGACGGAGGACTAGGGACTAACTGTTG

1486 CTCTTGGTGGCCTTGGCAGGGACTAGGCTGAAGATGTGCTTCTC

1531 CCCGCCACCTACTGTATGACACCACATTCTCCATAACAGCTGGG

1576 TTGTGAGGAATATGAAAAGAGCCTATTGATAGCTAGAAGGGAAAT

1621 ATGAAAAGGTAGAAGTGACTTCAAGGTACGGTACAGGTTCCCCCTCCAC

1666 CTCTGTACAGGCTTCTGACTACGTAGTTGGAGCTATTCTTCC

1711 CCCAGCAAAGGCCAGAGCTTGTCCCCGGCTCTGGACACATA

1756 GGGCATTATCTGTATTCTTGGCTGGCATCTTTAGCTCAGG

1801 AAGGTAGAAGAGATCTGTCCCCATGGGCTCTGGCTCAATCCC

1846 TTCTTGTTCAGTGACATATGTATTGTTATCTGGGTTAGGGATG

1891 GGGGACAGATAATAGAACCGAGCAAAGTAACCTATAACAGGCCAGCA

1936 TGAAACAGCATCTCCCCTGGCTTGTCTGGCTTGTGACGTAT

1981 AAGACAGAGCAGGCCACATGTGGCCATTCTGCTCCCCATTCTGA

2026 AAGCTGCTGGGCCTCTGCAAGGCTTCTGGATCTCTGGTCAGAG

2071 TGAACCTTGCTTCTGTATTCAAGGCACTCAGACAGAACAGTAA

2116 GGGGAGAGTCATACGTGTGGCCAGGAAGTAGCCAGGGTGAAGAG

2161 AGACTCGGTGCCGGCAGGGAGAATGCCCTGGGGTCCCTCACCTGG

2206 CTAGGGAGATACCGAAGCTACTGTGTAAGACTTCTGGGT

2251 TCTTCTCTGTCTGCTAACCCAGGGAGGGCTTAAGAGGAAGGTGAC

2296 TTCTCTGTCTTGTCTTAAGTTGCACTGGGGATTCTGACTTGA

2341 GGCCCATCTCTCCAGGCCACTGCCCTTGTAAATTAAAGT

2386 GCCTTGAGCTGGAATGGGAAGGGGACAAGGGTCAGTCTGTCGG

2431 GTGGGGGCAGAAATCAAATCAGCCCAAGGATATAAGTTAGGATTA

2476 TTACTTAATAGAGAAATCTAACTATATCACACAAAGGGATAACA

2521 CTATAAAATGTAATAAAATTATGTCTAGAAGTTAAAAAAAAAAAAA

2566 AAAAAAAGT

Figure 10. Clone 4004731_1

TTCATAACAAAATCCAGGCCAGGCACGGTGGCTCATTTTAAAGTCAAAAGAAAAAAT
 AGTACTTAAAAAATAGAAAAATAAAACTGTACACCAAAATAAGCTAGAAAATGGAAC
 TAAGAAATAATATTGAAATTAAATAAAATGAAGCTACAGAAGGCATAAGTAAGTCAA
 ATGTTGGCTTTGAAAGACTATTAAATAATTACACAGAAAGTCTAATAAGAGAAAAGA
 GAGAAAAAAACTGTCAGAATGCTACCGAAGTGTACTGCTTCTACAGTGAGAACACGGATC
 TGACTTGTGGCAGGCCAAGTGTGACAAGTCAATGCTGCCTATCCTCACCTGGCTCACC
 TGCCATCTGCCATGGCAGACTCATCCTCCGGTTCTCGCACATGGTGGCAGTCTGCGG
 MetAlaAspSerSerPheArgPheProArgThrTrpTrpGlnSerAlaGlu
 AGGATGTGCACAGAGAAAAGATCCAGTTAGACCTGGAAAGCTGAATTCTACTTCACTCACC
 AspValHisArgGluLysIleGlnLeuAspLeuGluAlaGluPheTyrPheThrHisLeu
 TAATTGTGATGTTCAAGTCCCCCAGGCCGCTGCCATGGTGGCTGGACCGCTCCAGGACT
 IleValMetPheLysSerProArgProAlaAlaMetValLeuAspArgSerGlnAspPhe
 TTGGGAAAACATGGAAGCCTTATAAGTACTTGCAGACTACTGCTCCGCTACATTTGGCC
 GlyLysThrTrpLysProTyrLysTyrPheAlaThrAsnCysSerAlaThrPheGlyLeu
 TGGAAAGATGATGTTGTCAAGAAGGGCGCTATTGTACTTCTAAATACTCCAGTCCTTTC
 GluAspAspValValLysLysGlyAlaIleCysThrSerLysTyrSerSerProPhePro
 CATGCACTGGAGGGAGAGGTATTTCAAAGCTTGTCAACCACCACGATACAGAGAAC
 CysThrGlyGlyGluValIlePheLysAlaLeuSerProProTyrAspThrGluAsnPro
 CTTACAGTGCCAAAGTTCAGGAGCAGCTGAAGATCACCAACCTCGCGTGCAGCTGCTGA
 TyrSerAlaLysValGlnGlnLeuLysIleThrAsnLeuArgValGlnLeuLys
 AACGACAGTCTGTCCCTGTCAGAGAAATGACCTGAACGAAGAGCCTCAACATTTACAC
 ArgGlnSerCysProCysGlnArgAsnAspLeuAsnGluGluProGlnHisPheThrHis
 ACTATGCAATCTATGATTCATTGTCAAGGGCAGCTGCTCTGCAATGCCACGCTGATC
 TyrAlaIleTyrAspPheIleValLysGlySerCysPheCysAsnGlyHisAlaAspGln
 AATGCATACTGTTCATGGCTTCAGACCTGTCAAGGCCAGGAACATTCCACATGGTCC
 CysIleProValHisGlyPheArgProValLysAlaProGlyThrPheHisMetValHis
 ATGGGAAGTGTATGTAAAGCACAACACAGCAGGCCACTGCCAGCAGTGTGCCCCGT
 GlyLysCysMetCysLysHisAsnThrAlaGlySerHisCysGlnHisCysAlaProLeu
 TATACAATGACCGGCCATGGAGGCAGCTGATGGAAAACGGGGCTCCAACGAGTGCA
 TyrAsnAspArgProTrpGluAlaAlaAspGlyLysThrGlyAlaProAsnGluCysArg
 GAACCTGCAAGTGTAAATGGCATGCTGATACCTGTCACTTGACGTTAATGTGTCGGAGG
 ThrCysLysCysAsnGlyHisAlaAspThrCysHisPheAspValAsnValTrpGluAla
 CATCAGGGAAATCGTAGTGGTGGTCTGTGATGACTGTCAGCACAACACAGAAGGACAGT
 SerGlyAsnArgSerGlyGlyValCysAspAspCysGlnHisAsnThrGluGlyGlnTyr
 ATTGCCAGAGGTGCAAGCCAGGCTTCTATCGTGACCTGCGGAGACCCCTCTCAGCTCCAG
 CysGlnArgCysLysProGlyPheTyrArgAspLeuArgArgProPheSerAlaProAsp
 ATGCTTGAAACGTAAGTAACTGTGGTTCCAGAAAATAGGCTGATTTGTACAAGAGAT
 AlaCysLysArgLys

GAATCTCTTATCCCTCATTCTGCTAACCCAAGAGAAGGGAGGTCAATTGAGGTTCTGAGAT
AACACACTTACAGATATCGGTTAATTCTTCATTGATAAGAAGCAAGAATTTCAACCAT
TGGGTGAAAACAGTATAATATTCATCAAAAGTAATTCCCTTCATTCTNCATAAAAGTA
AAAATTATTCCCTATACGCTGCATTTGGTAAACAGGGATGACTAATAGAAAAAAAAATGA
TGAANAAGGAGACTATTTAAGAACTTAAGACTACTTGGGAGTAGAAGGTAGACAATAATG
GACTCANCTGATGAAATAAAGGTAAGTACTGGACTTGGAAATATCTTACCTTACAGGGAA
CTTAAC

Figure 11. Clone 4009334_1

GCTCCGCGACTCGGCCTCTCCACCCCTCCCCAGCCTTCTCGCCCTCTTCTCCCACA
 CTCCCGGCCGGCGCTCGGTTGTGCGAGGAGATGGTGTAGCCCCCTGGCCGCCGAAGA
 GGAGCCGGACACTTGTCTCCGTCTCGAGCTGCTCCCCACCCCTGGAGGAGAGACCCCC
 CCCTCGGCTCGCGCCTCTCGGTCTCCGGCTGGTGGGGAAGCCTCTGCGCCGCCGGCA
 CCATGAGTGAACAGAGTATCTGTCAGGCAAGAGCTGCTGTATGGTTATGATGATGCCA
 MetSerGluGlnSerIleCysGlnAlaArgAlaAlaValMetValTyrAspAspAlaAsn
 ATAAGAAAGTGGGTGCCAGCTGGTGGCTCAACTGGATTCAAGCTGAGTTCATATCTATCACC
 LysLysTrpValProAlaGlySerThrGlyPheSerArgValHisIleTyrHisHis
 ATACAGGCAACAAACACATTCAAGAGTGGTGGGAGGAAGATTCAAGGACCATCAGGTCTGTA
 ThrGlyAsnAsnThrPheArgValValGlyArgLysIleGlnAspHisGlnValValIle
 TAAACTGTGCCATTCTAAAGGGTTGAAGTACAATCAAGCTACACAGACCTTCCACCAAGT
 AsnCysAlaIleProLysGlyLeuLysTyrAsnGlnAlaThrGlnThrPheHisGlnTrp
 GGCAGAGATGCTAGACAGGGTGTATGGTCTCAACTTGGCAGCAAAGAGGATGCCAATGTCT
 ArgAspAlaArgGlnValTyrGlyLeuAsnPheGlySerLysGluAspAlaAsnValPhe
 TCGCAAGTGCCATGATGCATGCCTTAGAAGTAAATTCAAGCTACAGAACGGCAACAT
 AlaSerAlaMetMetHisAlaLeuGluValLeuAsnSerGlnGluThrGlyProThrLeu
 TGCCTAGACAAAACCTACAACCTACCTGCTCAAGTCAAAATGGCCATCCAAAGAAGAAT
 ProArgGlnAsnSerGlnLeuProAlaGlnValGlnAsnGlyProSerGlnGluGluLeu
 TGGAAATTCAAAGAAGACAACATAAGAACAGCAACGGCAAAGGAGCTGGAGCGGGAAA
 GluIleGlnArgArgGlnLeuGlnGluGlnGlnArgGlnLysGluLeuGluArgGluArg
 GGCTGAAGCGAGAAAAGAATGAAAGAGAAAGGAAGAAGAGAGAGAGAGGTTAGAAAGGGAAA
 LeuLysArgGluArgMetGluArgGluArgLysArgGluArgLeuGluArgGluArg
 GGCTGGAGAGGGAGCGACTGAAACAAGAACAGCTGGAGAGAGAGAGACAAGAACGGGAAAC
 LeuGluArgGluArgLeuGluGlnGluGlnLeuGluArgGluArgGlnGluArgGluArg
 GGCAGGAACGCCCTGGAGCGCAGGAACGCCCTGGAGCGGAGGAACGCCCTGGAGCGGGCAGG
 GlnGluArgLeuGluArgGlnGluGluArgLeuGluArgGlnGluArgLeuGluArgGlnGlu
 AACGCCCTGGATCGGGAGAGGCAAGAAAGACAAGAACAGAGAGAGGGCTGGAGAGACTGGAAC
 ArgLeuAspArgGluArgGlnGluArgGlnGluArgGluArgLeuGluArgLeuGluArg
 GGGAGAGGCAAGAAAGGGAGCGACAAGAGCAGTTAGAAAGGGACAGCTGGAATGGGAGA
 GluArgGlnGluArgGluArgGlnGluLeuGluArgGluGlnLeuGluTrpGluArg
 GAGAGCGCAGAATATCAAGTGCTGCCCTGCCTCTGTTGAGACTCCTCTAAACTCTG
 GluArgArgIleSerSerAlaAlaAlaProAlaSerValGluThrProLeuAsnSerVal
 TGCTGGGAGACTCTCTGCTCTGAGCCAGGCTTGAGGAGCCCTCAGCCGGCCGAGA
 LeuGlyAspSerSerAlaSerGluProGlyLeuGlnAlaAlaSerGlnProAlaGluThr
 CTCCATCCAAACAGGAAGACAATGCCCTTAACGGACTTGCACTGCAATTGCCGGAG
 ProSerGlnGlnGluAspAsnArgProLeuThrGlyLeuAlaAlaIleAlaGlyAla
 CAAAACCTAGGAAAGTGTACGGATGGAGGATACCTCTTCCAAGTGGAGGGAATGCTA
 LysLeuArgLysValSerArgMetGluAspThrSerPheProSerGlyGlyAsnAlaIle
 TTGGTGTGAACTCCGCCTCATCTAAACAGATAACAGGCCGTGAAATGGACCCCTTC
 GlyValAsnSerAlaSerSerLysThrAspThrGlyArgGlyAsnGlyProLeuProLeu

TAGGGGGTAGTGGTTAACGAAAGAAATGAGTGCCCTGCTGGCACGAGGAGAAGAATTG
GlyGlySerGlyLeuMetGluGluMetSerAlaLeuLeuAlaThrArgArgArgIleAla

CTGAAAAGGGATCAACAATAGAACAGAACAAAAAGAGGACAAAGGTGAAGATTAGAGC
GluLysGlySerThrIleGluThrGluGlnLysGluAspLysGlyGluAspSerGluPro

CTGTAACCTCTAACCGCTCTTCACAAAGTACACCTGAACCAACAAAGAAAACCTTGGGAAA
ValThrSerLysAlaSerSerThrSerProGluProThrArgLysProTrpGluArg

GAACAAATACAATGAATGGCAGCAAGTCACCTGTTATCTCCAGACCTCCAAGGAAAAATC
ThrAsnThrMetAsnGlySerLysSerProValIleSerArgProProArgLysAsnGln

AGATTGTTTTGACAACAGGTCTATGATTCAATTACACAGACAAAATCCACACCCGTTA
IleValPheAspAsnArgSerTyrAspSerLeuHisArgProLysSerThrProValIle

TCACAGCCCAGTGCCAATGGAGTCCAGACGGAAGGACTTGACTATGACAGGCTGAAGCAG
ThrAlaGlnCysGlnTrpSerProAspGlyArgThr

GACATTTAGATGAAATGAGAAAAGAATTAACAAAGCTAAAAGAAGAGCTCATGGATGCA
ATCAGGCAGGAACTGAGCAAGTCAAATACTGCATAGAGGAACAGACTAAGGAGAGATAGG

ACTTTAATCTGGAGGAAAATATCCTACAAACAACAACACTGTTACAACAGCAAACCCCTA
CATTATGAGCTGTAAGAAGAAAATGGAGACAAACAGAAGGAGGGAAAACCAACCTACT

CTGAAAGCCTTCAGACATTATGACTCTGGTATAAGCTCTTCCCTCTCCGTTGCTGCT
TTTTCTGGCCAACATCAGAATGGTAACAC

Figure 12. Clone 4009334_2

GCTCCCGACTCGGCCTCTCACCCCCCTCCCCAGCCTTCTCTGCCCTCTCTCCCACA
 CTCCCGGCCGGCGCCTCGGCTTGCGAGGAGATGGTAGCCCCCTGGCCGCCAAGA
 GGAGCCGGACACTTGCTCCCGTCTCCGAGCTGCTCCCCACCCCTGGAGGAGAGACCCCC
 CCCTCGGCTCGGCCTTCTCGTCTCCGGCTGGTGGGAAGCCTCTGCGCCGCCGCA
 CCATGAGTGAACAGAGTATCTGTCAAGAGCTGCTGTGATGGTTATGATGATGCCA
 MetSerGluGlnSerIleCysGlnAlaArgAlaAlaValMetValTyrAspAspAlaAsn
 ATAAGAAGTGGGTGCCAGCTGGTGGCTCAACTGGATTCAAGCAGAGTTCATATCTATCACC
 LysLysTrpValProAlaGlyGlySerThrGlyPheSerArgValHisIleTyrHisHis
 ATACAGGCAACACACATTCAAGAGTGGTGGCAGGAAGATTCAAGGACCACAGGTGTA
 ThrGlyAsnAsnThrPheArgValValGlyArgLysIleGlnAspHisGlnValValIle
 TAAACTGTGCCATTCTAAAGGGTTGAAGTACAATCAAGCTACACAGACCTTCCACCAGT
 AsnCysAlaIleProLysGlyLeuLysTyrAsnGlnAlaThrGlnThrPheHisGlnTrp
 GCGCAGATGCTAGACAGGTGTATGGTCTCAACTTGGCAGCAAAGAGGATGCCAATGTCT
 ArgAspAlaArgGlnValTyrGlyLeuAsnPheGlySerLysGluAspAlaAsnValPhe
 TCGCAAGTGCCTATGATGCATGCCTTAGAAGTGTAAATTCACAGGAAACAGGGCAACAT
 AlaSerAlaMetMetHisAlaLeuGluValLeuAsnSerGlnGluThrGlyProThrLeu
 TGCTTAGACAAAACACTACAACACTACCTGCTCAAGTTCAAAATGGCCCATCCCAAGAAGAAT
 ProArgGlnAsnSerGlnLeuProAlaGlnValGlnAsnGlyProSerGlnGluGluLeu
 TGGAAATTCAAAGAACAACTACAAGAACAGCAACGGCAAAAGGAGCTGGAGCGGGAAA
 GluIleGlnArgArgGlnLeuGlnGlnArgGlnLysGluLeuGluArgGluArg
 GGCTGAAGCGAGAAAGAACATGGAAAGAGAAAGGAAGAACAGAGAGAGAGAGGTTAGAAAGGGAAA
 LeuLysArgGluArgMetGluArgLysLysArgGluArgLeuGluArgGluArg
 GGCTGGAGAGGGAGCGACTGGAACAAGAACAGCTGGAGAGAGAGAGACAAGAACGGGAAC
 LeuGluArgGluArgLeuGluGlnGluGlnLeuGluArgGluArgGlnGluArgGluArg
 GGCAGGAACGCCTGGAGCGCAGGAACGCCTGGAGCGCAGGAACGCCTGGAGCGCAGG
 GlnGluArgLeuGluArgGlnGluArgLeuGluArgGlnGluArgLeuGluArgGlnGlu
 AACGCCTGGATCGGGAGAGGCAAGAAAGAACAGAACAGAGAGAGAGACTGGAAAC
 ArgLeuAspArgGluArgGlnGluArgGlnGluArgGluArgLeuGluArgLeuGluArg
 GGGAGAGGCAAGAAAGGGAGCGACAAGAGCAGCTTAGAAAGGGAACAGCTGGAATGGGAGA
 GluArgGlnGluArgGluArgGlnGluGlnLeuGluArgGluGlnLeuGluTrpGluArg
 GAGAGCGCAGAATATCAAGTGTGCTGCCCTGCCTCTGTTGAGACTCCTCTAAACTCTG
 GluArgArgIleSerSerAlaAlaAlaProAlaSerValGluThrProLeuAsnSerVal
 TGCTGGGAGACTCTCTGCTCTGAGCCAGGCTTGCAGGCAGCCTCTCAGCCGGCGAGA
 LeuGlyAspSerSerAlaSerGluProGlyLeuGlnAlaAlaSerGlnProAlaGluThr
 CTCCATCCAACAGGAAGACAATGCCCTTAACTGGACTTGCAGCTGCAATTGCCGGAG
 ProSerGlnGlnGluAspAsnArgProLeuThrGlyLeuAlaAlaAlaIleAlaGlyAla
 CAAACTTAGGAAAGTGTACGGATGGAGGATACTCTTCCCAAGTGGAGGGAAATGCTA
 LysLeuArgLysValSerArgMetGluAspThrSerPheProSerGlyGlyAsnAlaIle

TTGGGTGTGAACTCCGCCTCATCTAAAACAGATAACAGGCCGTGGAAATGGACCCCTTCCTT
GlyValAsnSerAlaSerSerLysThrAspThrGlyArgGlyAsnGlyProLeuProLeu

TAGGGGGTAGTGTTAATGGAAGAAATGAGTGCCCTGCTGGCACGAGGAGAAGAATTG
GlyGlySerGlyLeuMetGluGluMetSerAlaLeuLeuAlaThrArgArgArgIleAla

CTGAAAAGGGATCAACAATAGAACAGAACAAAAAGAGGACAAAGGTGAAGATTGAGC
GluLysGlySerThrIleGluThrGlnLysGluAspLysGlyGluAspSerGluPro

CTGTAACCTCTAAGGCCTCTCAACAAGTACACCTGAACCAACAAGAAAACCTGGGAAA
ValThrSerLysAlaSerSerThrSerThrProGluProThrArgLysProTrpGluArg

GAACAAATACAATGAATGGCAGCAAGTCACCTGTTATCTCCAGACCAAAATCCACACCC
ThrAsnThrMetAsnGlySerSerProValIleSerArgProLysSerThrProLeu

TATCACAGCCCAGTGCCAATGGAGTCCAGACGGAAGGACTTGACTATGACAGGCTGAAGC
SerGlnProSerAlaAsnGlyValGlnThrGluGlyLeuAspTyrAspArgLeuLysGln

AGGACATTTAGATGAAATGAGAAAAGAATTAACAAAGCTAAAGAGCTCATTGATG
AspIleLeuAspGluMetArgLysGluLeuThrLysLeuLysGluLeuIleAspAla

CAATCAGGCAGGAACTGAGCAAGTCAAATACTGCATAGAGGAACAGACTAAGGAGAGATA
IleArgGlnGluLeuSerLysSerAsnThrAla

GGACTTTAATCTGGAGGAAAAATATCCTACAAACAACTGTTCACACAGCAAACCCCC
TACATTTATGAGCTGTAAGAAGAAAATGGAGACAAACAGAAGGAGGGAAAAACCAACCTA
CTCTGAAAGCCTTCAGACATTATGACTCTGGTATAAGCTTTCCCTCTCCGTTGCTG
CTTTTTCTGGCCAACATCAGAATGGTAACAC

Figure 13. Clone 4035508

GAATTCGAGCGCAGGAGCTCCGTTCTCACCTGCTCCGGGGAGCTAGTGGGATCCAGA
GAATCACCCGCTGATGGTTTGCCCAGGCCTGAAACAACCAGAGAGCTACGGAAAGGA
AGGGCTTGGCTGCCAGAGGAATTCCAAGTGCTCAAACGCCAGGCTACGGCGCTGT
GATCCGTCCAGGAGGACAAAGTGGGATTGAGGATCCACTCCACTCTGCTCATGGCG
M A R
CCAGGGCCTGCCCTGCACGTGGCCACACTGCTGACTGGGCTGCTGGAATGCCCTGGGCTT
Q G L P L H V A T L L T G L L E C L G F
TGCTGGCGTCCTCTTGCTGGCTTCACTAGTGTGTTGCTCAAGAACGATTACTT
A G V L F G W P S L V F V F K N E D Y F
TAAGGATCTGTGGACCAGATGCTGGGCCATTGGCAATGCCACAGGGCAGGCTGACTG
K D L C G P D A G P I G N A T G Q A D C
CAAAGCCCAGGATGAGAGGTTCTCACTCATCTTCACCCCTGGGTCTCATGAAACAAC
T K A Q D E R F S L I F T L G S F M N N F
CATGACATTCCCCACTGGCTACATCTTGACCGGTTCAAGACCACCGTGGCACGCC
M T F P T G Y I F D R F K T T V A R L I
AGCCATATTTCTACACCAACGCCACACTCATAGCCTTCACCTCTGCAGCTTCTT
A I F F Y T T A T L I I A F T S A A S L
ATGAAAAAGGCATCAGCCTCAGGGCTCCTCATCTCATCTGTCTGCAAGTACCTGG
CATGTAGCACGCACCTTCCTCTGATGCCCGGGGACATCCCACCCACTGCC
AACTACAGCTATGCCCTGTGCCCTGGGAATGGCACCAAGGAAGAGAAGGAAACAGCT
GAGCATGAAAACAGGGAGCTACAGTCAAAGGAGTTCTTCAGCGAA

Figure 14. Clone 4339264

CTTTTTTCAGATAACATCTTGTGAGTCATAACCAGCCTGGTCCCCCATGATCGTGGGGTCCCTCGGGCCTGACACAG
Met Ile Val Gly Ser Pro Arg Ala Leu Thr Gln

CCCCCTGGGTCTCCCTCGCCTGCTGCAGCTGGTGTCTACCTGCGTGGCCTTCTCGCTGGTGGCTAGCGTGGGCCCTGGAC
Pro Leu Gly Leu Leu Arg Leu Leu Glu Leu Val Ser Thr Cys Val Ala A Phe Ser Leu Val Ala Ser Val Gly Ala Trp Th

GGGGTCCATGGGCAACTGGTCCATGTTCACCTGGTCTCTGCTTCTCCGTGACCCCTGATCATCCTCATCGTGGAGCTGT
r Gly Ser Met Gly Asn Trp Ser Met Phe Thr Trp Cys Phe Cys Phe Ser Val Thr Leu Ile Ile Leu Ile Val Glu Leu C

GCGGGCTCCAGCCCCGCTTCCCCCTGCTTGGCGCAACTTCCCCATCACCTTCGCTATCGGGCCCTCTCTGCCCTC
ys Gly Leu Gln Ala Arg Phe Pro Leu Ser Trp Arg Asn Phe Pro Ile Thr Phe Ala Cys Tyr Ala Ala Leu Phe Cys Leu

TCGGCCTCCATCATCTACCCCACCACCATATGTCAGCTCTGTCACGGCCGTTGCGGGACCACGCCATGCCGCC
Ser Ala Ser Ile Ile Tyr Pro Thr Thr Tyr Val Gln Phe Leu Ser His Gly Arg Ser Arg Asp His Al a Ile Ala Ala Th

CTTCTTCTCCTGCATCGCGTGTGGCTTACGCCACCGAAGTGGCCTGGACCCGGCCGGCCGGCGAGATCACTGGCT
r Phe Phe Ser Cys Ile Ala Cys Val Ala Tyr Ala Thr Glu Val Ala Trp Thr Arg Ala Arg Pro Gly Glu Ile Thr Gly T

ATATGGCCACCGTACCCGGCTGCTGAAGGTGCTGGAGACCTTCGCTTGCGTGCATCATCTTCGCGTTCATCGC
yr Met Ala Thr Val Pro Gly Leu Leu Lys Val Leu Glu Thr Phe Val Ala Cys Ile Ile Phe Ala A Phe Ile Ser Asp Pro

AACCTGTACCAGCACCAGCCGCCCTGGAGTGGTGGCGGTACGCCATCTGCTTCACTCTAGCGGCCATGCC
Asn Leu Tyr Gln His Gln Pro Ala Leu Glu Trp Cys Val Ala Val Ile Cys Phe Ile Leu Ala Al a Ile Ala Ile Ala Ile

CCTGCTGAACCTGGGGAGTGCACCAACGTGCTACCCATCCCCTCCCCAGCTTCTGTCGGGGCTGGCCTGCTGCTG
e Leu Leu Asn Leu Gly Glu Cys Thr Asn Val Leu Pro Ile Pro Phe Pro Ser Phe Leu Ser Gly Leu Ala Leu Leu Ser V

TCCTCCTCATGCCACCGCCCTGTTCTGGCCCTCTACCAGTCGATGAGAAAGTATGGCGCCAGCCTCGCGCTCG
a Ile Leu Tyr Ala Thr Ala Leu Val Leu Trp Pro Leu Tyr Gln Phe Asp Glu Lys Tyr Gly Gln Pro Arg Arg Ser

AGAGATGTAAGCTGCAGCCGCAGCCATGCCTACTACGTGTGCGCTGGGACCGCCGACTGGCTGTGGCCATCCTGACGGC
Arg Asp Val Ser Cys Ser Arg Ser His Ala Tyr Tyr Val Cys Ala Trp Asp Arg Arg Leu Ala Val Al a Ile Leu Thr Al

CATCACCACTACTGGCGTATGGCTGACCTGGTGCACCTGCCCACCTGGTTTGTCAGGTCTAAAGACTCTCCAAGA
a Ile Asn Leu Leu Ala Tyr Val Ala Asp Leu Val His Ser Ala His Leu Val Phe Val Lys Val

GGCTCCCGTTCCCTCTCCAACCTCTTGTTCTGCCCAGTTCTTATGGAGTACTTCTTCCTCCGCTTCC

CTGTTTCTCTCCGTCTCC

Figure 15. pCR2.1-2864933

1 GGATCCGGTTCCCAGAAGATTCTGAGCCAATCAGTATTTCGCAT
 GlySerGlyPheProGluAspSerGluProIleSerIleSerHis
 46 GGCAACTATAACAAAACAGTATCCGGTGTGGGCCACAAGCCA
 GlyAsnTyrThrLysGlnTyrProValPheValGlyHisLysPro
 91 GGACGGAACACCACACAGAGGCACAGGCTGGACATCCAGATGATT
 GlyArgAsnThrThrGlnArgHisArgLeuAspIleGlnMetIle
 136 ATGATCATGAACGGAACCCCTCACATTGCTGCTAGGGACCATT
 MetIleMetAsnGlyThrLeuTyrIleAlaAlaArgAspHisIle
 181 TATACTGTTGATATAGACACATCACACACGGAAAGAAATTATTGT
 TyrThrValAspIleAspThrSerHisThrGluGluIleTyrCys
 226 AGCAAAAAACTGACATGGAAATCTAGACAGGCCGATGTAGACACA
 SerLysLysLeuThrTrpLysSerArgGlnAlaAspValAspThr
 271 TGCAGAACATGAAGGGAAAACATAAGGGATGAGTGCCACAACATT
 CysArgMetLysGlyLysHisLysAspGluCysHisAsnPheIle
 316 AAAGTTCTCTAAAGAAAAACGATGATGCATTGTTGTCTGTGGA
 LysValLeuLeuLysLysAsnAspAspAlaLeuPheValCysGly
 361 ACTAATGCCCTCAACCCCTCCTGCAGAAACTATAAGATGGATACA
 ThrAsnAlaPheAsnProSerCysArgAsnTyrLysMetAspThr
 406 TTGGAACCATTGGGGATGAATTCAAGCGGAATGCCAGATGCCA
 LeuGluProPheGlyAspGluPheSerGlyMetAlaArgCysPro
 451 TATGATGCCAACATGCCAACGTTGCACTGTTGCAGATGGAAAA
 TyrAspAlaLysHisAlaAsnValAlaLeuPheAlaAspGlyLys
 496 CTATACTCAGCCACAGTGACTGACTTCCTGCCATTGACCGAGTC
 LeuTyrSerAlaThrValAspPheLeuAlaIleAspAlaVal
 541 ATTTTACCGGAGTCTGGAGAAAGCCCTACCCCTGCGGACCGTCAAG
 IleTyrArgSerLeuGlyGluSerProThrLeuArgThrValLys
 586 CACGATTCAAATGGTTGAAAGAACCATACTTGTCAAGCCGTG
 HisAspSerLysTrpLeuLysGluProTyrPheValGlnAlaVal
 631 GATTACGGAGATTATCTACTTCTTCAGGGAAATAGCAGTG
 AspTyrGlyAspTyrIleTyrPhePhePheArgGluIleAlaVal
 676 GAGTATAACACCAGGGAAAGGTAGTTTCCAAGAGTGCTCAG
 GluTyrAsnThrMetGlyLysValValPheProArgValAlaGln
 721 GTTTGTAAGAATGATATGGGAGGATCTCAAAGAGTCCTGGAGAAA
 ValCysLysAsnAspMetGlyGlySerGlnArgValLeuGluLys
 766 CAGTGGACGTCGTCCTGAAGGCGCGCTTGAAGTGCCTCAGTT
 GlnTrpThrSerPheLeuLysAlaArgLeuAsnCysSerValPro
 811 GGAGACTCTCATTTTATTCAACATTCTCCAGGCAGTTACAGAT
 GlyAspSerHisPheTyrPheAsnIleLeuGlnAlaValThrAsp
 856 GTGATTGTATCAACGGGCGTGATGTTGTCTGGCAACGTTTCT
 ValIleArgIleAsnGlyArgAspValValLeuAlaThrPheSer

901 ACACCTTATAACAGCATCCCTGGGTCTGCAGTCTGTGCCTATGAC
ThrProTyrAsnSerIleProGlySerAlaValCysAlaTyrAsp

946 ATGCTTGACATTGCCAGTGTGTTACTGGGAGATTCAAGGAACAG
MetLeuAspIleAlaSerValPheThrGlyArgPheLysGln

991 AAGTCTCCTGATTCCACCTGGACACCAGTCCTGATGAACGAGTT
LysSerProAspSerThrTrpThrProValProAspGluArgVal

1036 CCTAAGCCCAGGCCAGGTTGCTGTGCTGGCTCATCCTCCTAGAA
ProLysProArgProGlyCysCysAlaGlySerSerLeuGlu

1081 AGATATGCAACCTCCAATGAGTTCCCTGATGATGATAACCTGAACCTC
ArgTyrAlaThrSerAsnGluPheProAspAspThrLeuAsnPhe

1126 ATCAAGACGCACCCGCTCATGGATGAGGCAGTGCCCTCCATCTTC
IleLysThrHisProLeuMetAspGluAlaValProSerIlePhe

1171 AACAGGCCATGGTTCCCTGAGAACAAATGGTCAGATAACGCCCTTAC
AsnArgProTrpPheLeuArgThrMetValArgTyrArgLeuThr

1216 AAAATTGCGAGTGGACACAGCTGCTGGGCCATATCAGAATCACACT
LysIleAlaValAspThrAlaAlaGlyProTyrGlnAsnHisThr

1261 GTGGTTTTCTGGGATCAGAGAACGGGAATCATCTTGAAGTTTTG
ValValPheLeuGlySerGluLysGlyIleIleLeuLysPheLeu

1306 GCCAGAAATAGGAAATAGTGGTTTCTAAATGACAGCCTTTCTG
AlaArgIleGlyAsnSerGlyPheLeuAsnAspSerLeuPheLeu

1351 GAGGAGATGAGTGTAACTCTGAAAAATGCAGCTATGATGGA
GluGluMetSerValTyrAsnSerGluLysCysSerTyrAspGly

1396 GTCGAAGACAAAAGGATCATGGGATGCAAGCTGGACAGAGCAAGC
ValGluAspLysArgIleMetGlyMetGlnLeuAspArgAlaSer

1441 AGCTCTCTGTATGTTGCGTTCTCACCTGTGTGATAAGGTTCCC
SerSerLeuTyrValAlaPheSerThrCysValIleLysValPro

1486 CTTGGCCGGTGTGAACGACATGGGAAGTGTAAAAAACCTGTATT
LeuGlyArgCysGluArgHisGlyLysCysLysThrCysIle

1531 GCCTCCAGAGACCCATATTGTGGATGGATAAAGGAAGGTGGTGCC
AlaSerArgAspProTyrCysGlyTrpIleLysGluGlyGlyAla

1576 TGCAGCCATTATCACCCAACAGCAGACTGACTGAGCAGGAC
CysSerHisLeuSerProAsnSerArgLeuThrPheGluGlnAsp

1621 ATAGAGCGTGGCAATACAGATGGTCTGGGGACTGTCACAATTCC
IleGluArgGlyAsnThrAspGlyLeuGlyAspCysHisAsnSer

1666 TTTGTGGCACTGAATGGCATTCCAGTTCCCTCTTGCCCCAGCACA
PheValAlaLeuAsnGlyHisSerSerLeuLeuProSerThr

1711 ACCACATCAGATTGACGGCTCAAGAGGGTATGAGTCTAGGGGA
ThrThrSerAspSerThrAlaGlnGluGlyTyrGluSerArgGly

1756 GGAATGCTGGACTGGAAGCATTGCTTGACTCACCTGACAGCACA
GlyMetLeuAspTrpLysHisLeuLeuAspSerProAspSerThr

1801 GACCCTTGGGGCAGTGTCTTCCATAATCACCAAGACAAGAAG
AspProLeuGlyAlaValSerSerHisAsnHisGlnAspLysLys

1846 GGAGTGATTGGGAAAGTTACCTCAAAGGCCACGACCAGCTCGAG
GlyValIleArgGluSerTyrLeuLysGlyHisAspGlnLeuGlu

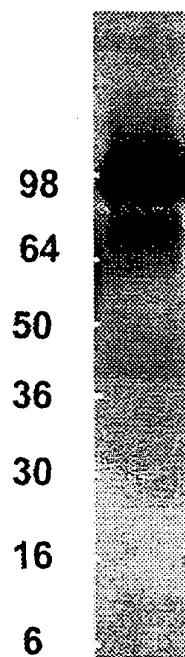


Figure 16. Human 2864933 protein secreted by 293 cells.

Figure 17, Panel A.

GGATCC AACTGCCCTCCGTCTGCTCGTGCAGTAACCAGTCAG
 CAAGGTGGTGTGCACGCGCCGGGCCTCTCGAGGTCCCGCAGGGTATTCCCTCGAACACCCGGTACCTAACCTCATGG
 AGAACAAACATCCAGATGATCCAGGCCGACACCTTCCGCCACCTCCACCACCTGGAGGTCCCTGCAGTTGGGAGGAACCTCC
 ATCCCGCAGATTGAGGTGGGGCCTTCACACGGCCCTGGCAAGCCTCAACACCCCTGGAGCTGTTGACAACACTGGCTGACAGT
 CATCCCTAGCGGGGCCTTGAAATAACCTGTCCAAGCTGCGGGAGCTCTGGCTTCGAACAAACCCCATCGAAAGCATCCCC
 CTTACGTCTCAACCGGGTGCCTCCCTCATGCGCTGGACTTGGGGAGCTCAAGAAGCTGGAGTATATCTGAGGG
 GCTTTGAGGGGCTGTTCAACCTCAAGTATCTGAACACTGGGATGTGCAACATTAAAGACATGCCCAATCTCACCCCC
 GGTGGGGCTGGAGGAGCTGGAGATGTCAGGAAACACTCCCTGAGATCAGGCCCTGGCTCCTCCATGCCCTGAGCTCCC
 TCAAGAAGCTCGGTATGAACTCACAGTCAGCCTGATTGAGCAGGAAATGCTTTGACGGGCTGGCTTCACTTGTGGAA
 CTCAACTTGGCCACAATAACCTCTTCTTGCCTCATGACCTTTACCCGCTGAGGTACCTGGTGGAGTTGCATCT
 ACACCCAACCCCTTGAACACTGTGATTGTGACATTCTGTGGTAGCCTGGCTGGCTTCGAGAGTATATAACCAACCAATTCA
 CCTGCTGTGGCCGCTGTATGCTCCCATGCACATGCGAGGCCCTACCTCGTGGAGGTGGACCCAGGCCCTTCCAGTGC
 TCTGCCCCCTTCATCATGGACGCACCTCGAGACCTCAACATTCTGAGGGCTGGATGGCAGAACTTAAGTGTGGACTCC
 CCCTATGTCCTCCGTGAAGTGGTGTGCTGCCAATGGGACAGTGTCAAGCCATGCCCTCCGCCACCAAGGATCTGTCC
 TCAACGACGGCACCTGAACCTTCCCACGTGCTGCTTCAAGACACCGGGGTGTACACATGCATGGTGACCAATGTTGCA
 GGCAACTCCAACGCTCGGCCTACCTCAATGTGAGCACGGCTGAGCTTAACACCTCAAACAGCTTCTACCACAGT
 AACAGTGGAGACCGAGATTCGCTGAGGACACAACGCGAAAGTACAAGCCTGTTCTACCACGTCCACTGGTTACC
 AGCCGGCATATACCACCTTACCAACGGTCGAG

Figure 17, Panel B.

NCPSVCSNSQFSKVVCTRGLSEVPQGIPSNTRYLNLMENNIQMIQADTFRLHHLEVLQLGRNSIRQIEVGAFNG
 LASLNTLELFDNWLTVIPSGAFEYLSKLRELWLRNNPIESIPSYAFNRVPSLMRLDLGELKKLEYISEGAFEGLFNL
 KYLNLMGNIKDMPNLTPLVGLEELEMMSGNHFPEIRPGSFHGLSSLKKLWVMNSQVSLIERNAFDGLASLVELNLAH
 NNLSSLPHDLFPLRYLVELLHHNPWNCDCDILWLAWWLREYIPTNSTCCGRCHAPMHMRGRYLVEVDQASFQCSA
 PFIMDAPRDLNISEGRMAELKCRTPPMSSVKWLLPNGTVLSHASRHPRISVLNDGTLNFSHVLLSDTGVYTCMTNV
 AGNSNASAYLNVSTAELNTSNYSFFTTVETTEISPEDTRKYKPVPTTSTGYQPAYTTSTT

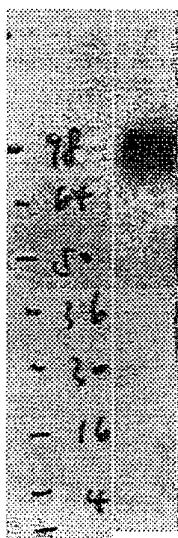


Figure 18. Human 3352358 protein secreted by 293 cells.

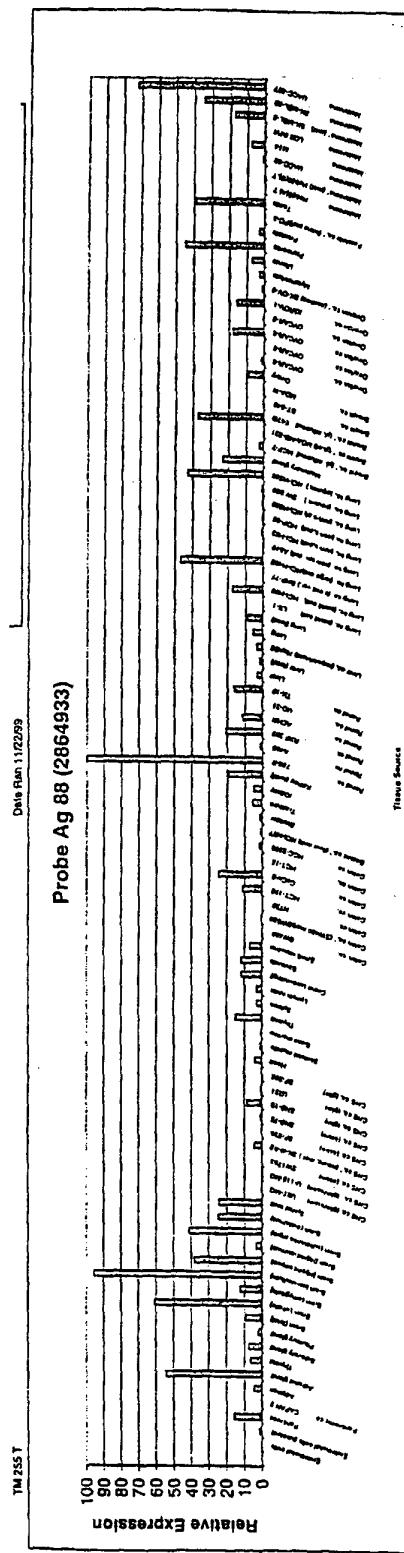


FIG. 19A

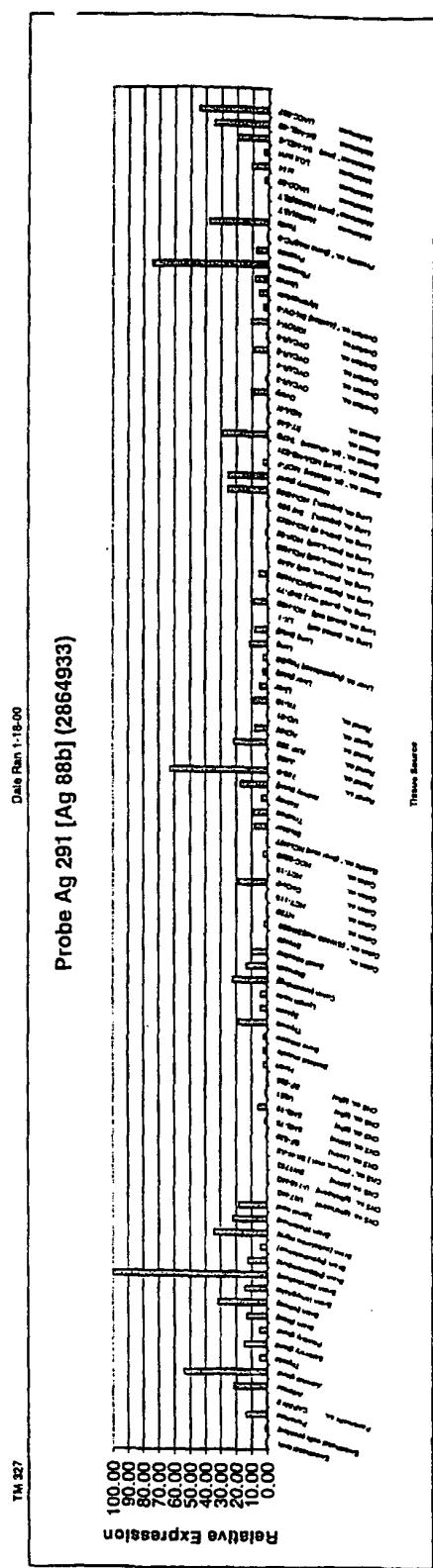


FIG. 19B

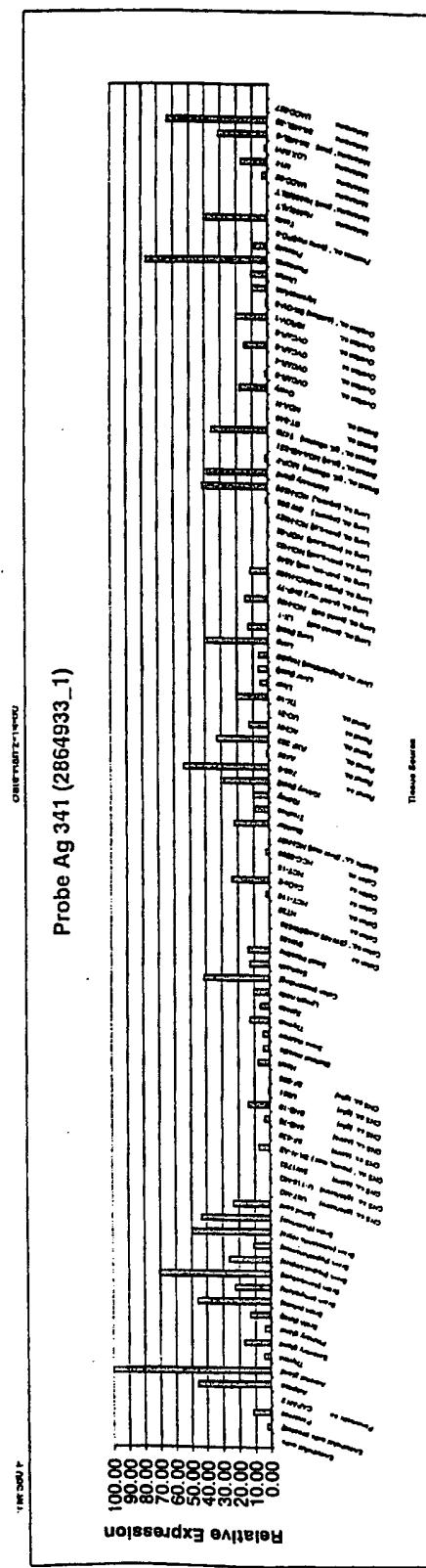


FIG. 19C

tm293f_ag42f

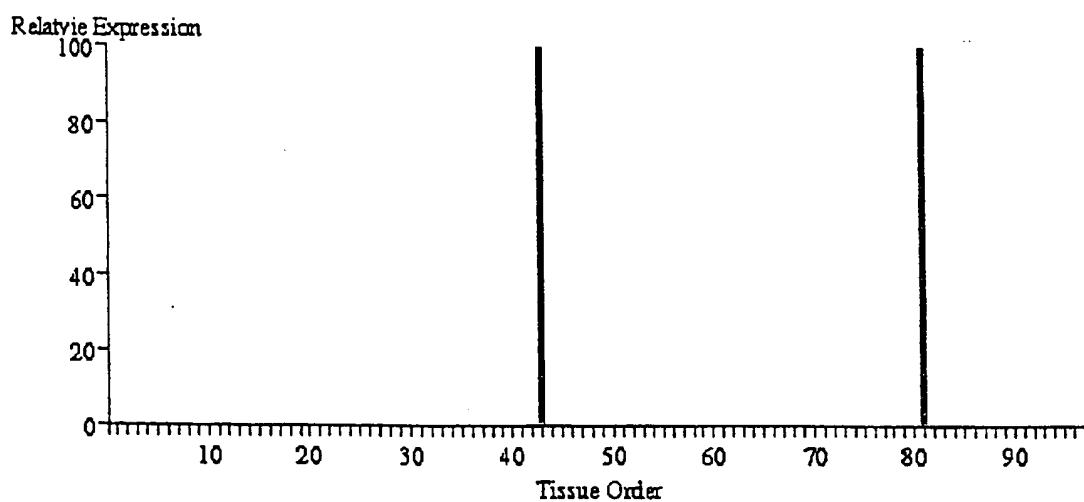


FIG. 20

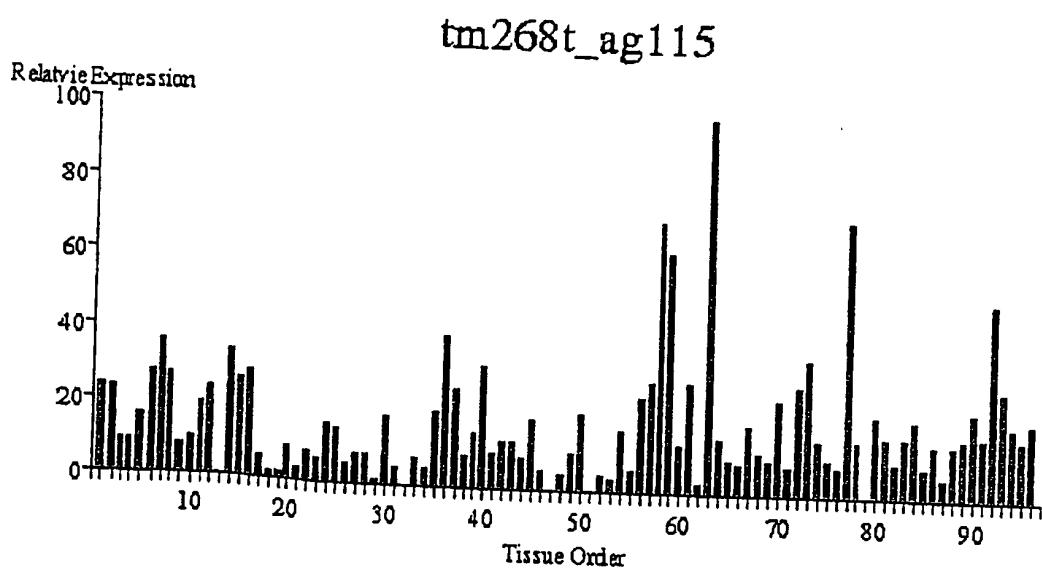


FIG. 21

tm264f_ag118

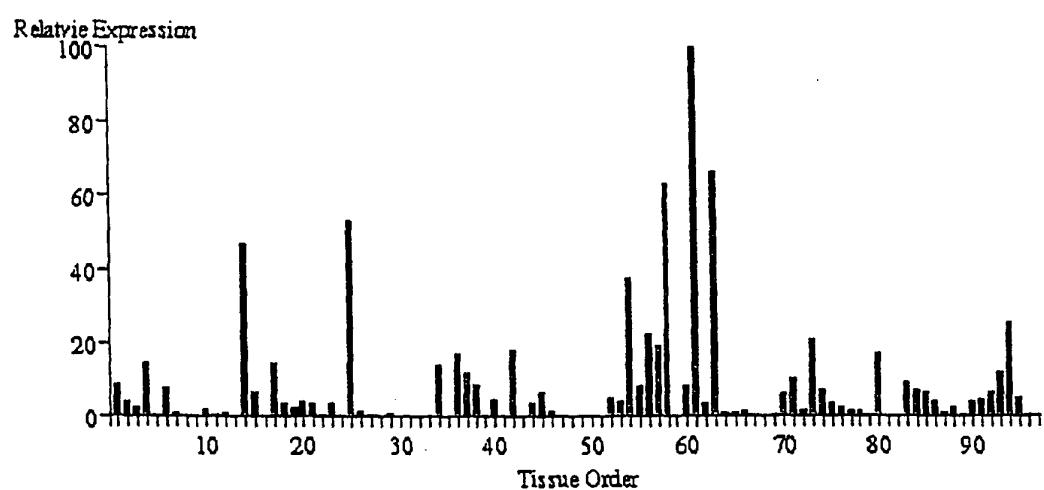


FIG. 22

tm266f_ag120

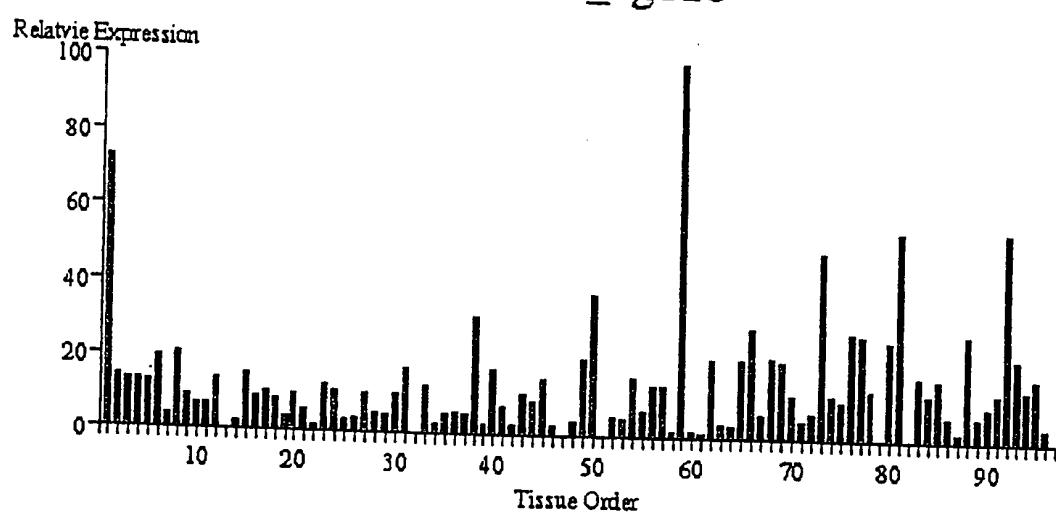


FIG. 23

